Systemic delivery of ST317, a cell penetrating STING pathway sensitizer, results in strong anti-tumor activity

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STipe Therapeutics

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Background

STING is a transmembrane protein found at the endoplasmic reticulum (ER) and is a key regulator of early innate immune responses against pathogens. STING is broadly expressed in both immune and non-immune cells but it has been reported to be less active in the tumor micro-environment (TME) through epigenetic silencing or through hypoxia-dependent inhibition.
Pharmacological activation of the STING pathway is currently being assessed as a strategy to trigger an anti-tumor immune response in so-called "cold" tumors, as its activation in tumor cells and/or antigen presenting cells in the tumor microenvironment can induce production of proinflammatory cytokines (e.g. Type I IFN) and chemokines (e.g. CXCL10). Such overall activation results in the induction and recruitment of cells that drive the innate and adaptive systems, mediating tumor clearance.

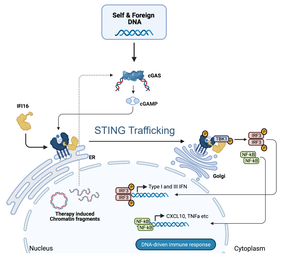
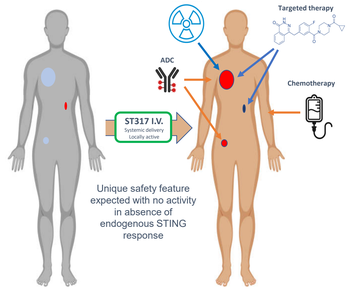


Figure 1: Double-stranded DNA (dsDNA)-induced activation of the cGAS-STING pathway.

Although the first generation of STING agonists dosed intratumorally (IT) demonstrated a strong anti-tumor response in preclinical models, positive clinical data in metastatic patients has so far been limited, potentially due to poor distribution, even within injected tumors. Newer classes of STING agonists that are delivered systemically are expected to give more consistent and predictable levels of STING activation; but this may not be as well tolerated due to peripheral activation of the immune system resulting in immune related adverse events. With this in mind, STipe Therapeutics is developing a **STING sensitizer, called ST317, that decreases the threshold at which STING agonists trigger an anti-tumor immune response. ST317 is safe when systemically administrated and will sensitize tumors and innate immune cells to STING activation** when combined with therapies known to locally induce suboptimal STING pathway activation (STING agonists, Radiation, ADC, ISAC etc…).



ST317 **potentiates the immunological benefit of cytoplasmic DNA** observed in tumors with high mutation loads/CIN

ST317 **reduces the threshold** necessary for STING pathway activation by cGAMP, while **locally inducing** supraphysiological levels of pro-inflammatory cytokines/chemokines in the TME.

Combination partners (e.g. radiation, PARP inhibitors, ADC, ISAC and to a lesser extent chemotherapies) that will locally increase cytoplasmic DNA/cGAMP production

ST317: a STING pathway sensitizer

We have previously demonstrated that Interferon gamma inducible factor 16 (IFI16) promotes activation of the cGAMP-activated STING-TBK1 pathway by a process relying on IFI16 PYRIN domain-dependent protein-protein interactions (1).

Figure 2: Schematic structure of the STING sensitizer ST317

ST317 is a linear peptide whose sequence is partially derived from IFI16 and also contains a cell penetrating peptide (CPP) and which mimics some of the biological functions of IFI16.
ST317 acts as a sensitizer of the cGAS-STING pathway, leading to enhanced responsiveness to STING activation, without acting as an agonist itself.

ST317 characteristics

- Reduces threshold at which STING pathway is activated and potentiates the pathway
- Poorly immunogenic; systemic delivery should be well tolerated
- Preferentially sensitizes STING where the pathway is sub-optimally active
- Can be combined with agents that preferentially induce STING in the TME (Local irradiation/radionuclide, PARP inhibitor, chemotherapy, ADC, ISAC etc…)

References: (1) IFI16 is required for DNA sensing in human macrophages by promoting production and function of cGAMP. Jønsson KL, et al Nat Commun. 2017 Feb 10;8:14391. doi: 10.1038/ncomms14391. PMID: 28186168.
(2) Design of amidobenzimidazole STING receptor agonists with systemic activity. Ramanjulu JM et al. Nature. 2018 Dec;564(7736):439-443. doi: 10.1038/s41586-018-0705-y. PMID: 30405246

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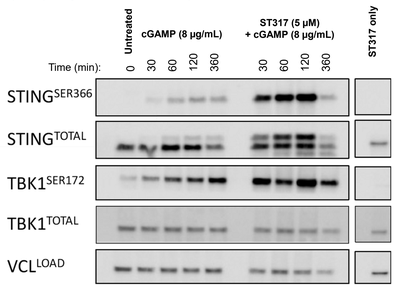
ST317 enhances STING pathway activation and trafficking to the Golgi

STING activation in response to cGAMP binding results in increased STING, TBK and IRF3 phosphorylation, which is dependent on translocation of STING from the ER to Golgi.



In the presence of cGAMP (or other agonists, not shown), ST317 specifically enhances STING activation as shown by increased phosphorylation of STING and TBK1. ST317 also increased the duration of STING pathway activation. Without an endogenous STING agonist (e.g. cGAMP), ST317 does not trigger phosphorylation of STING and TBK1.

Figure 3: Western blot analysis of PMA-differentiated THP1 cells after cGAMP stimulation and ST317 sensitization. Unstimulated (lane 1); lanes 2-10: stimulated with 2'3'-cGAMP without (lanes 2-5) or with ST317 (lanes 7-10). Last lane shows THP1 cells treated with ST317 only. Treatment times are indicated (minutes).

ST317 combined with cGAMP significantly increases STING trafficking from the ER to Golgi which was observed less than 10 min after treatment with the combination (albeit not yet significant at this timepoint). Reciprocally, STING residing in ER is significantly reduced after 1 hour of treatment with ST317/cGAMP. Single agent ST317 did not affect STING trafficking.

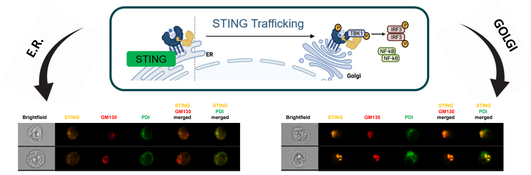


Figure 4: Representative images of colocalized STING with ER and GOLGI markers.

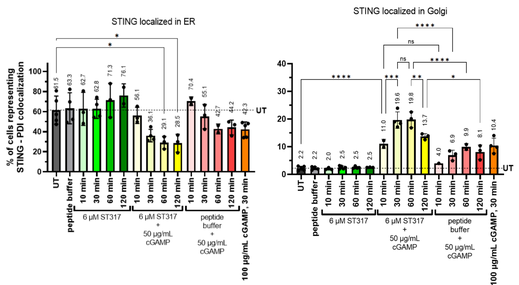


Figure 5: STING localization in the ER and the Golgi. The PMA-differentiated THP1 cells were untreated or treated with the peptide buffer alone, ST317, cGAMP or a combination of both. Cells were then stained for STING, GM130 (Golgi marker) and PDI (ER marker). Around 20,000 cells were acquired on ImageStream® (n= up to 5 independent experiments +/- SD.)

IT delivery of ST317 sensitizes MC38 tumours to suboptimal dose of ADU-S100 *in vivo*

Single agent IT delivery of either ST317 or a suboptimal dose of ADU-S100 did not activate the STING pathway in MC38 TME. The combination of both resulted in STING activation, as shown by induction of pSTING, pTBK1 and pIRF3.

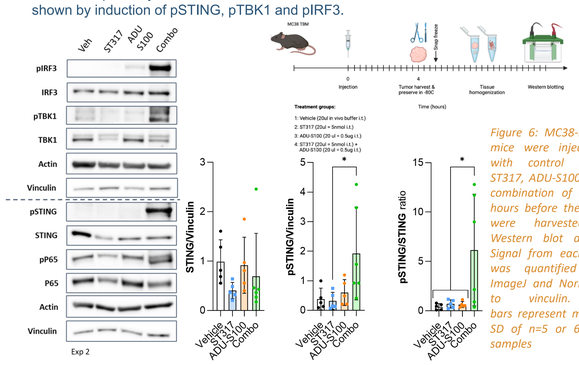


Figure 6: MC38-bearing mice were injected IT with control vehicle, ST317, ADU-S100 or the combination of both 4 hours before the tumor were harvested for Western blot analysis. Signal from each band was quantified using ImageJ and Normalized to vinculin. The bars represent means ± SD of n=5 or 6 lysate samples

ST317 sensitizes THP1 cells and PBMCs to STING agonists therapy

ST317 sensitizes THP1 cells to cGAMP. The combination reduced the EC_{50} and/or increased the E_{max} for CXCL10 (and to a lesser extent on type I IFN). High concentrations of cGAMP had a limited negative effect on THP1 viability but this was not further affected by addition of ST317. Similar sensitization potential was observed to other STING agonists (ADU-S100 and diABZi Compound 3).

THP1

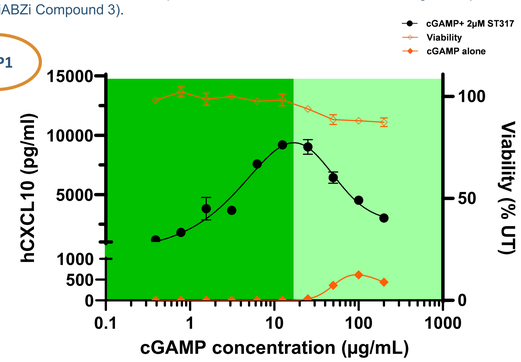


Figure 7: CXCL10 release upon stimulation and sensitization with cGAMP +/- ST317. Undifferentiated THP1 cells were treated with cGAMP for 30 min prior to sensitization with or without 2 µM ST317. After incubation for twenty hours, supernatants were collected and analyzed for CXCL10 (representative of n=4). Data represent means ± SD. Cell viability data for the combination at 20hr is included for comparison of cGAMP effect.

	2´3´-cGAMP (n=4)							
	CXCL10 6h	CXCL10 6h (+ST317)	CXCL10 20h	CXCL10 20h (+ST317)	Type I IFN 6h	Type I IFN 6h (+ST317)	Type I IFN 20h	Type I IFN 20h (+ST317)
EC_{50} (µg/mL)	43.6 ± 7.8	nd	29.8 ± 16	2.7 ± 1.2	nd	25.3 ± 23.7	nd	20.9 ± 13.2
E_{Max} (pg/mL)	73.4 ± 4.8	726 ± 377	2169 ± 1774	21459 ± 11073	1633 ± 608	1949 ± 1729	1425 ± 1315	615 ± 307

Table 1: Summary table of cGAMP EC_{50} and E_{Max} values based on CXCL10 and Type I IFN response at two different timepoints (6 and 20 h, mean ± SD), n=4. nd: not determined.

PBMC

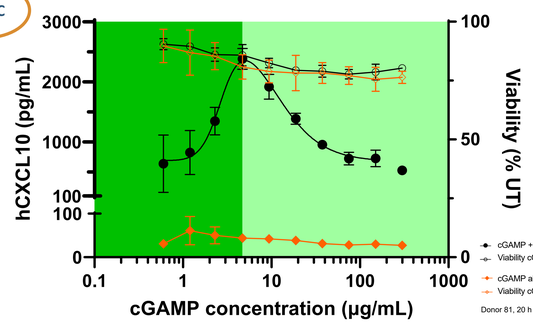


Figure 8: CXCL10 release upon stimulation and sensitization with cGAMP +/- ST317. Freshly isolated PBMCs were stimulated with cGAMP for 30 min prior to sensitization with or without 1 µM ST317. At twenty-hours supernatants were collected and analyze for human CXCL10 (representative of n=3). Data represents means ± SD. Cell viability data for the combination at 20hr is included for comparison of cGAMP effect.

ST317 sensitizes freshly isolated PBMC cells to non-cyclic dinucleotides such as diABZI compound 3 [2]

PBMC

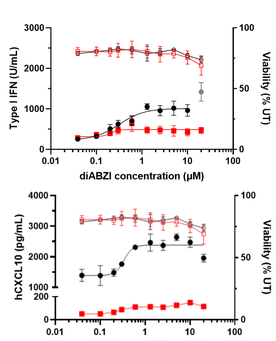
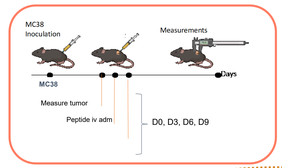


Figure 9: Type I IFN and CXCL10 release upon stimulation with diABZI compound 3 and sensitization +/- ST317. PBMCs were stimulated with diABZI C3 for 30 min prior to sensitization with or without 1 µM ST317 for 20 hrs. Type I IFN and CXCL10 were then quantified. Cell viability data for the combination at 20hr is included for comparison of diABZI C3 effect. Similar data were obtained with ADU-S100 (not shown).

ST317 delivered IV triggers tumor growth inhibition as single agent in an immuno-competent model

The ST317 single agent treatment groups exhibited significant anti-tumor activity compared to vehicle control against MC38 syngeneic efficacy model (representative of 2 independent in vivo experiments). No detrimental effects on animal weight were observed in response to ST317 (not shown)



Group	(n)	Tx	Dose level	RoA	Dosing freq. and duration
1	10	Vehicle	-	i.v.	Q3Dx4
2	10	ST317	10 mg/kg BW	i.v.	Q3Dx4
3	10	ST317	20 mg/kg BW	i.v.	Q3Dx4

i.v administration of ST317 by a slow release (>30 sec) of a volume =/< 150µL.

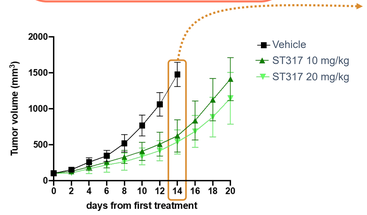
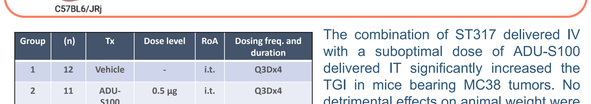


Figure 10: In vivo efficacy following systemic IV delivery of ST317 (10 and 20 mg/kg) in the MC38 subcutaneous syngeneic tumor model in C57BL/6 mice.

ST317 delivered IV sensitizes MC38 syngeneic tumors to suboptimal STING agonist *in vivo*



Group	(n)	Tx	Dose level	RoA	Dosing freq. and duration
1	12	Vehicle	-	i.t.	Q3Dx4
2	11	ADU-S100	0.5 µg	i.t.	Q3Dx4
3	12	ST317	10 mg/kg BW	i.v.	Q3Dx6
4	11	Combo	0.5 µg 10 mg/kg BW	i.t. i.v.	Q3Dx4 Q3Dx6

The combination of ST317 delivered IV with a suboptimal dose of ADU-S100 delivered IT significantly increased the TGI in mice bearing MC38 tumors. No detrimental effects on animal weight were observed in response to the different treatments (representative of 2 independent in vivo experiments).

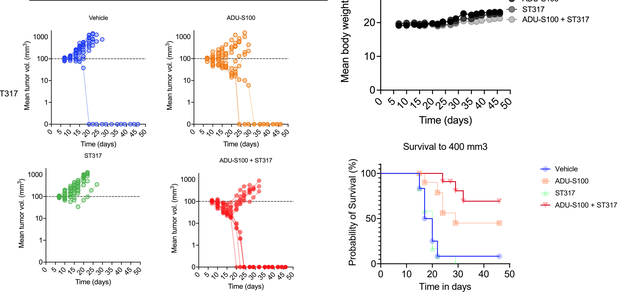


Figure 11: In vivo efficacy following systemic IV delivery of ST317 and IT delivery of suboptimal ADU-S100 in the MC38 subcutaneous syngeneic tumor model.

ST317 summary and conclusions

ST317 is a first-in-class STING sensitizer, which enhances STING pathway activation only at sites at which STING agonists are present. Importantly, ST317 is suitable for systemic delivery.

In vitro, ST317 reduces the EC_{50} at which agonists activate STING while inducing supraphysiological (increases the E_{max}) levels of pro-inflammatory cytokines/chemokines (e.g. Type I interferon and CXCL10)

ST317 increases and accelerates STING translocation from the ER to the Golgi in response to agonist, resulting in earlier and longer activation of the STING pathway.

ST317 is safe for repeated systemic administration in rodents and NHPs. ST317 i.v. dosing in tumor bearing mice was associated with anti-tumor efficacy when used in combination with suboptimal dose STING agonist (ADU-S100)

IND/CTA-enabling studies and CMC activities ongoing. The data warrant the assessment of ST317 as single agent and in combination in cancer patients Clinical Trials (H1-2024)