Generation of an antibody-drug conjugate-optimized TLR7/8 agonist payload

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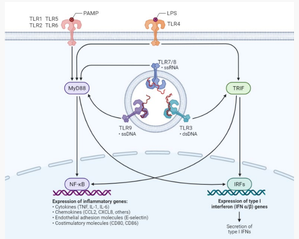
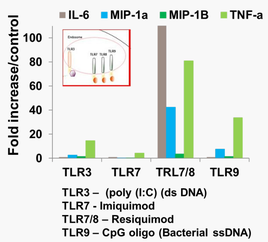
Developing ADCs that target endosomal TLRs

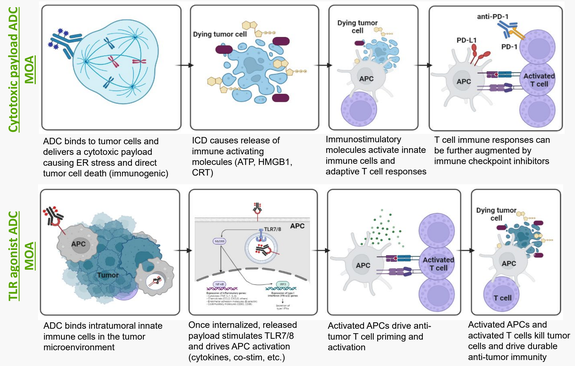
- Toll-like receptor (TLR) agonists stimulate innate immune cells and prime downstream T cell activation to drive potent and lasting anti-tumor immunity.
- Systemically administered TLR agonists have limited clinical use due to toxicities.
- Antibody drug conjugates are a clinically validated drug platform that delivers drugs to targeted cells with improved efficacy and tolerability.
- We developed a TLR7/8 agonist with physical and biological properties optimized as an ideal ADC payload.
- The payload combined with an optimized drug linker and immune targeting mAb drove durable anti-tumor activity in several syngeneic tumor models.





Dual TLR7/8 agonists are superior at re-programming tumor associated macrophages (TAMs). Intracellular pattern recognition receptors include TLR3, TLR9 and TLR7/8, can sense danger signals and translate these signals downstream through transcriptional modulation to activate an innate and subsequent adaptive immune response. In vitro derived human tumor associated macrophage (TAM)-like cells (IL-10 primed macrophages) are potently activated, demonstrated through increased cytokine induction, using a dual TLR7 and TLR8 agonist.

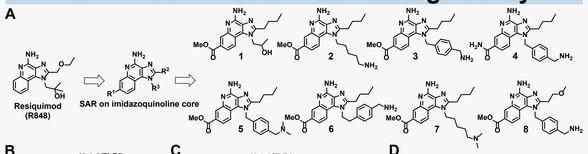
TLR7/8 ADC Proposed Mechanisms of Action

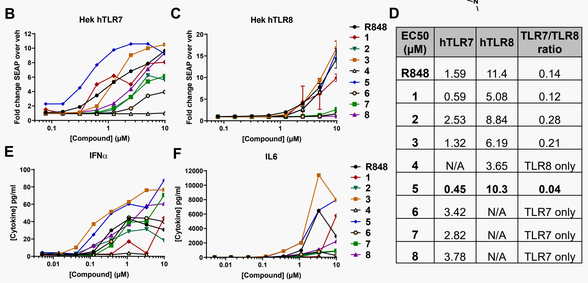


TLR7/8 ADCs have a differentiated MOA from traditional cytotoxic payload ADCs. Traditional cytotoxic payload ADCs drive direct tumor cell killing, often associated with passive immune stimulation, which can further be combined with immune checkpoint blockade. Conversely, a TLR7/8 agonist payload works directly through activation of intra-tumoral immune cells which drives APC activation and subsequent T cell priming. This coordinated immune cell activation can drive tumor cell killing with a durable anti-tumor immunity.

Results

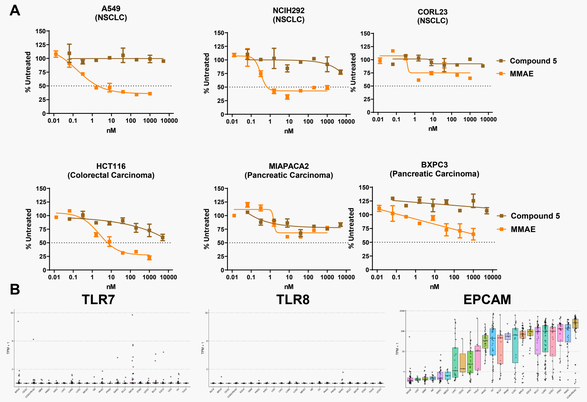
Novel Imidazoquinoline-based TLR7/8 agonists have robust immune stimulating activity





EC50 (μM)	hTLR7	hTLR8	TLR7/TLR8 ratio
R848	1.59	11.4	0.14
1	0.59	5.08	0.12
2	2.53	8.84	0.28
3	1.32	6.19	0.21
4	N/A	3.65	TLR8 only
5	0.45	10.3	0.04
6	3.42	N/A	TLR7 only
7	2.82	N/A	TLR7 only
8	3.78	N/A	TLR7 only

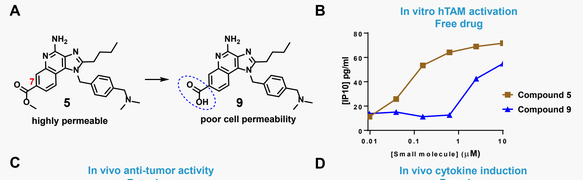
Imidazoquinolines with distinct structures drive differential TLR7 and TLR8 agonism and immune activation. A. A selected set of new imidazoquinoline compounds. **B-D**. Activation of Hek cells expressing TLR7 (**B**) or TLR8 (**C**) (Invivogen), with half-maximal effective concentrations (EC50) for each compound in each cell line and the TLR7 vs. TLR8 ratio delineated in the table (**D**). **E-F**. Activation of human PBMCs, delineated with IFNα (**E**) and IL6 (**F**) production, in response to various agonists. Data are mean of three different donors.

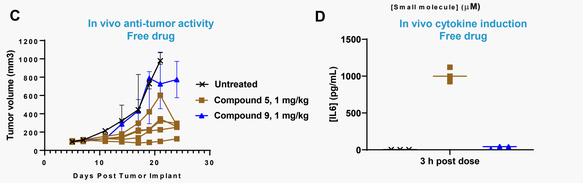


Lead TLR7/8 agonist, compound 5, does not drive direct tumor cytotoxicity or promote tumor cell growth. A) In several varied solid tumor cell lines, treatment with the lead TLR7/8 agonist did not drive appreciable cytotoxicity (compared with the microtubule disrupting cytotoxic payload MMAE) or cell growth as has been occasionally reported[#]. **B)** Lack of activity in these solid tumor cell lines are in line with low to no expression of TLR7 or TLR8 across a wide range of solid tumor cell lines (RNAseq data from CCLE; a classical tumor target EPCAM is included as a comparator).

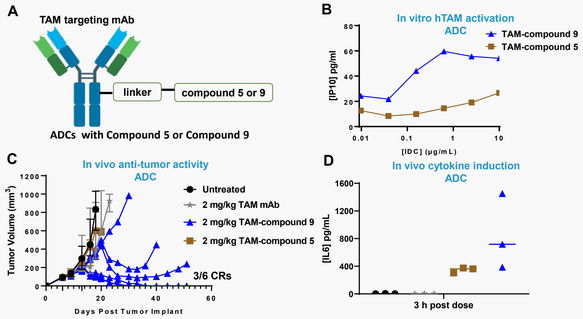
[#] Chatterjee et al. *Cancer Res.*2014; 74(18): 5008–5018; Grimmig et al. Int J Oncol. 2015; 47(3): 857–866.

Creation of an ADC optimized TLR7/8 agonist

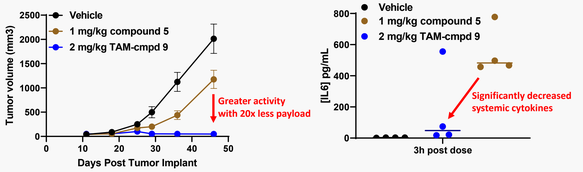




Chemical hydrolysis of compound 5 led to less permeable and less potent compound 9. A) Compound 5 was modified at the C7 position to create a less permeable compound 9 as measured by the MDCK II assay. (**B**) Immune activation, in hTAM-like cells, was decreased by the less permeable compound 9 vs the highly active compound 5 as measured by cytokine induction. Decreased anti-tumor activity was also seen for compound 9 in a murine Renca tumor model study (**C**) and in systemic cytokine induction for those animals (**D**)

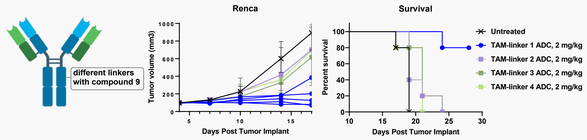


Linkage of the less permeable compound 9 to a TAM-targeting antibody enhances immune simulation and anti-tumor activity. A) Compounds 5 or 9 were linked to a TAM targeting antibody. The ADC activity of compound 9, both cytokine induction in vitro from human TAM-like cells (**B**) and in vivo in Renca tumors (**C, D**) was greatly increased when delivered directly via an ADC approach.

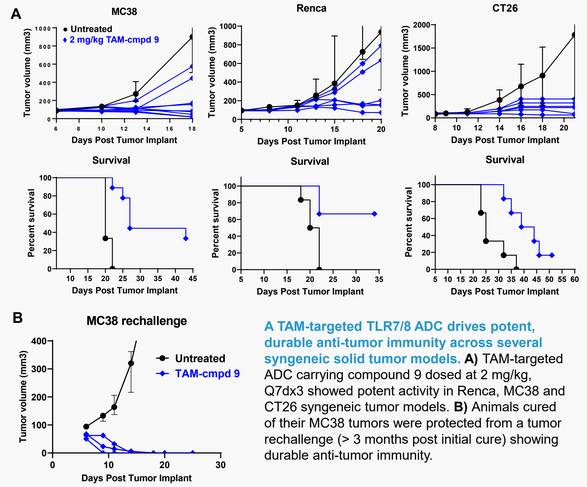


Superior in vivo efficacy and diminished systemic cytokine production were seen with the TAM targeting ADC carrying the optimized payload. MC38 colorectal cancer model was used to evaluate the efficacy and cytokine induction ability between small molecule compound 5 and TAM-targeting ADC with compound 9.

Optimized TLR7/8 agonist ADC has potent, durable antitumor activity



Linker components largely impact the in vivo efficacy of TLR7/8 ADC. Linker optimization was performed, and linker variations were assessed in vivo in the Renca renal syngeneic tumor model, dosed at 2 mg/kg Q7dx3. Linker 1 on the TAM-targeted mAb demonstrated superior anti-tumor activity.



A TAM-targeted TLR7/8 ADC drives potent, durable anti-tumor immunity across several syngeneic solid tumor models. A) TAM-targeted ADC carrying compound 9 dosed at 2 mg/kg, Q7dx3 showed potent activity in Renca, MC38 and CT26 syngeneic tumor models. **B)** Animals cured of their MC38 tumors were protected from a tumor rechallenge (> 3 months post initial cure) showing durable anti-tumor immunity.

Conclusions

- A novel set of TLR7/8 agonists were developed with various TLR7 and TLR8 agonistic activities and abilities to activate immune cells.
- An ADC-optimized TLR7/8 agonist payload was developed that leveraged differential permeability and enhanced activity through the TAM-targeted drug delivery.
- An optimized TLR7/8 agonist delivered to TAMs via an ADC drove durable, potent activity across several syngeneic tumor models.
- The reduced cytokine induction in vivo with TAM targeting ADCs can potentially provide better safety profile than systemically administered small molecule TLR7/8 agonist.
- These data collectively demonstrate that rational chemical design can create new payloads to release the full potential of the ADC targeted delivery concept.

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Disclosure

*Authors are or were Seagen employees at the time of data generation and hold stock in Seagen

^Work conducted while at Seagen but no longer currently employed at Seagen

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