Optimization of purine-based TLR7 agonists as payloads for immune-stimulating antibody conjugates (ISACs)

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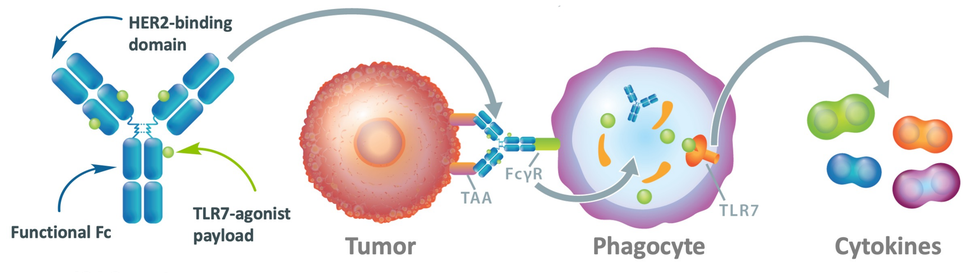
ISACs deliver immunostimulatory payloads to tumors to exert immune-mediated anticancer activity

Immune-stimulating antibody conjugates (ISACs) consist of antibodies conjugated to immuno-stimulatory small molecules and are designed to induce antitumor immune response. Systemic toxicities and lack of efficacy have hampered recent ISAC clinical development, providing an incentive to identify novel payloads. Substituted purines have previously been identified to elicit TLR7 activation. Here, we demonstrate that newly designed purine-based TLR7 agonists conjugated to trastuzumab show significant tumor volume reduction in a HER2-high gastric cancer xenograft model without associated body weight loss (BWL).



ISAC anatomy

- HER2 binding
- Fc-competent (IgG1)
- TLR7-agonist payload

1	2	3
HER2-binding domain engages tumor-associated antigen (TAA)	Fc-region engages phagocyte, leading to internalization and payload release	TLR7-agonism induces cytokine expression, promotes innate and adaptive immune response

Small molecules were screened and selected for ISAC conjugation based on potency and structural diversity

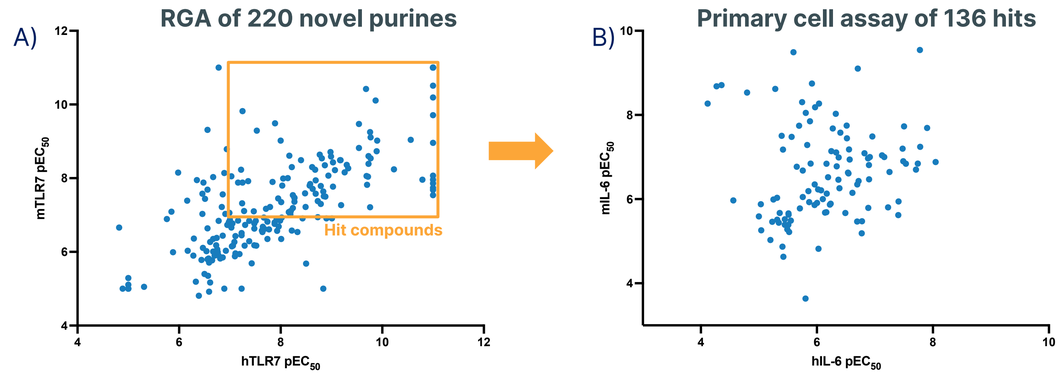
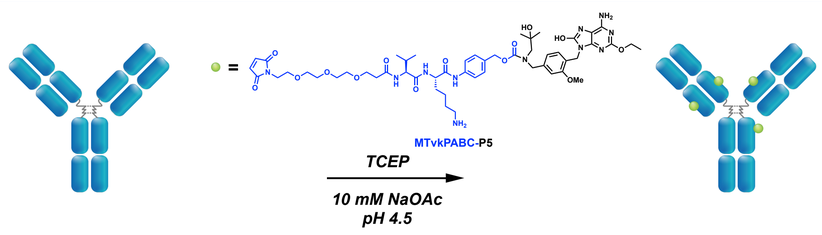


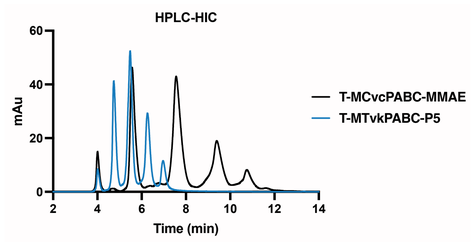
Figure 1. Response from (A) TLR7 reporter gene assay (RGA) or (B) IL-6 induction from primary cells. Human results on X-axis and murine on Y-axis. pEC_{50} = $-log_{10}(EC_{50})$.

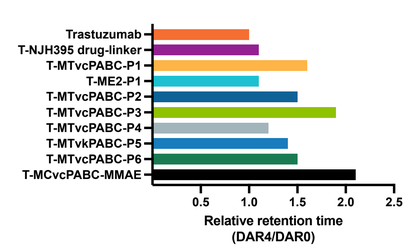
- 220 novel small molecule purines, purposefully designed for antibody conjugation, were synthesized and screened for their ability to agonize TLR7 using reporter gene assays
- Hit compounds (pEC_{50} > 7 in both assays) were followed up with screening human and murine primary immune cells
- Drug-linkers were generated from the 40 most promising compounds using cleavable and noncleavable linkers

Novel purine drug-linkers generate ISACs with favorable biophysical characteristics



- ISACs were generated through reaction of maleimide-bearing drug-linkers to the endogenous interchain disulfides on trastuzumab to achieve an average drug-to-antibody ratio (DAR) of 4
- P1, P2, P3, etc. are novel purine payloads





- The resulting ISACs demonstrated low aggregation and were comparably less hydrophobic than a trastuzumab DAR4 MMAE conjugate

Purine ISACs drive potent immune response in human and mouse co-culture systems

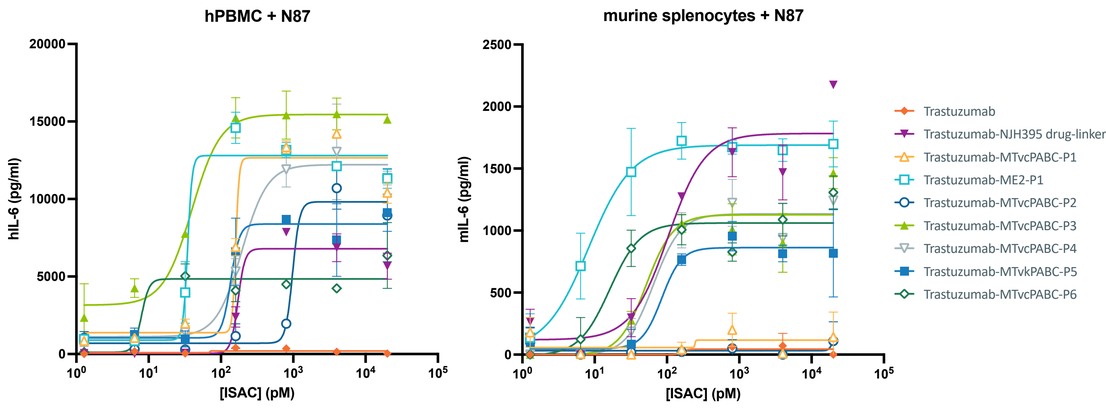


Figure 2. Human PBMCs or murine splenocytes were co-cultured with $HER2^+$ N87 tumor cells in the presence of indicated ISACs. Supernatant was collected after 72 h and 48 h for human PBMCs and mouse splenocytes, respectively, and IL-6 was assessed by homogeneous time resolved fluorescence (HTRF). No activation of immune cells was observed in the absence of tumor cells at the concentrations tested (data not shown).

- ISACs capable of inducing high levels of IL-6 from cocultures of tumor cells and primary immune cells were selected for in vivo studies

Novel ISACs show competitive in vivo efficacy to trastuzumab-NJH395 drug-linker

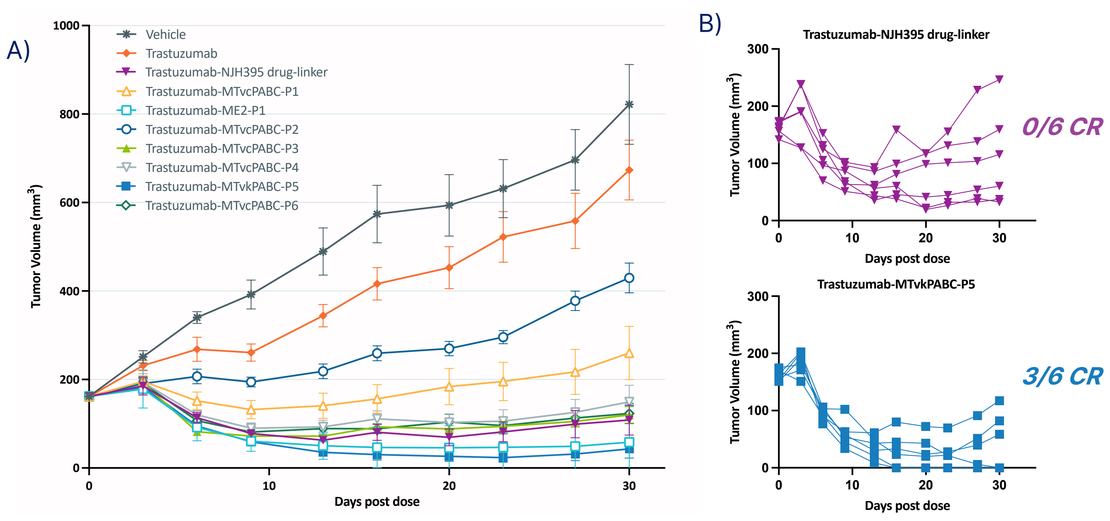


Figure 3. (A) Antitumor activity of purine-based TLR7 agonists conjugated to trastuzumab (DAR = 4) in an NCI-N87 tumor cell-line derived xenograft BALB/c nude model (6 mice per group), following single intravenous administration of 2.5 mg/kg of the respective ISACs or control articles. (B) Individual animal measurements reveal complete responses (CR) in the MTvcPABC-P5 group. PK analysis showed comparable exposure across the test articles (data not shown)

Novel ISACs show superior in vivo tolerability to trastuzumab-NJH395 drug-linker

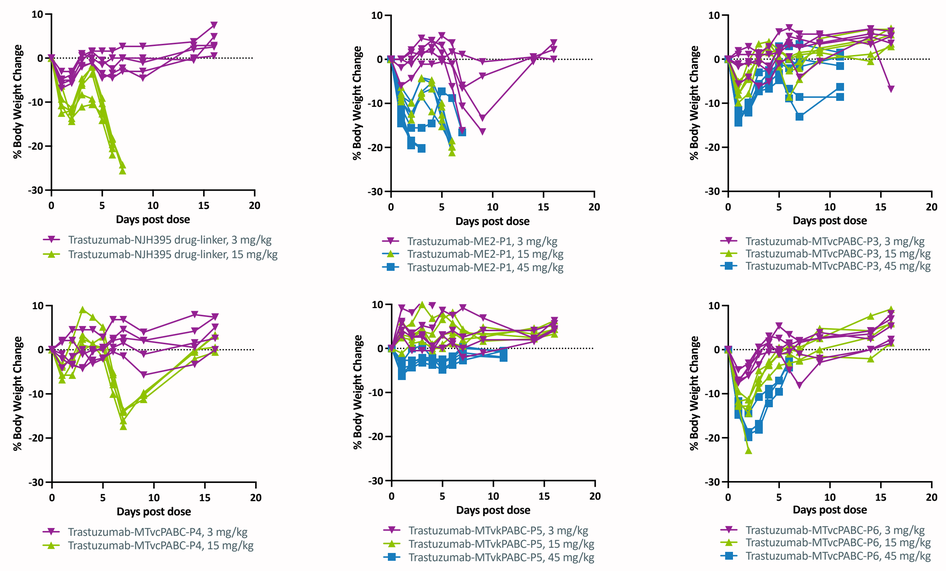


Figure 4. Body weight change over time of healthy BALB/c mice following single intravenous administration of 3, 15, or 45 mg/kg of the respective ISACs or control articles. In contrast to trastuzumab conjugated with NJH395 drug-linker, ISACs bearing the MTvkPABC-P5 and MTvcPABC-P3 drug-linkers were well tolerated even when administered at 18x their efficacious dose. MTD was not reached for these test articles in this study.

In vivo tumor growth rate inhibition (TGRI) correlates with in vitro immune response

- Correlations between in vivo TGRI and several in vitro metrics were investigated to improve our ability to select the most promising drug-linkers during the screening process
- A general correlation was observed between in vitro IL-6 response and TGRI, although certain compounds were noted outliers
- In vivo tumor growth rate inhibition (TGRI) was calculated according to the following formula:

$$\mathrm{TGRI} = [1 - \frac{tumor\ growth\ rate\ kinetic\ of\ treated\ group}{tumor\ growth\ rate\ kinetic\ of\ control\ group}]\ x\ 100$$

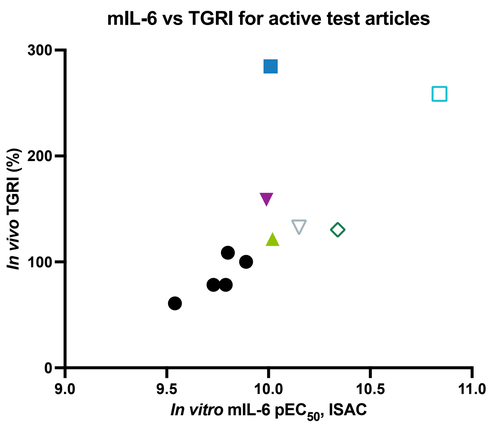


Figure 5. In vivo TGRI plotted against in vitro mIL-6 from ISAC coculture assay. Black circles represent test articles omitted from Figure 3.

Conclusions

220 purines screened in RGA → 136 selected for primary cell assay → 40 ISACs generated → 12 in vivo candidates selected → 5 ISACs evaluated for tolerability

- 220 purine small molecules were generated and evaluated for TLR7-agonism. The most promising 40 compounds were conjugated to trastuzumab as a model antibody system and the corresponding ISACs demonstrated favorable biophysical properties
- Purine-based ISACs showed strong activity on both murine and human immune cells in vitro. This cross-species conservation of activity negates the use of surrogate molecules for in vivo studies, providing greater translational relevance than other platforms
- In vivo efficacy studies in an N87 xenograft model indicate activity comparable or superior to the clinically evaluated NJH395 drug-linker
- Tolerability studies in BALB/c mice suggest trastuzumab conjugated with our lead drug-linker has significant tolerability advantage compared to trastuzumab conjugated to the benchmark NJH395 drug-linker
- Zymeworks is open to partnerships to accelerate the development of this program

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