zymeworks

Evaluation of TLR7-agonists as payloads for immune-stimulating antibody conjugates

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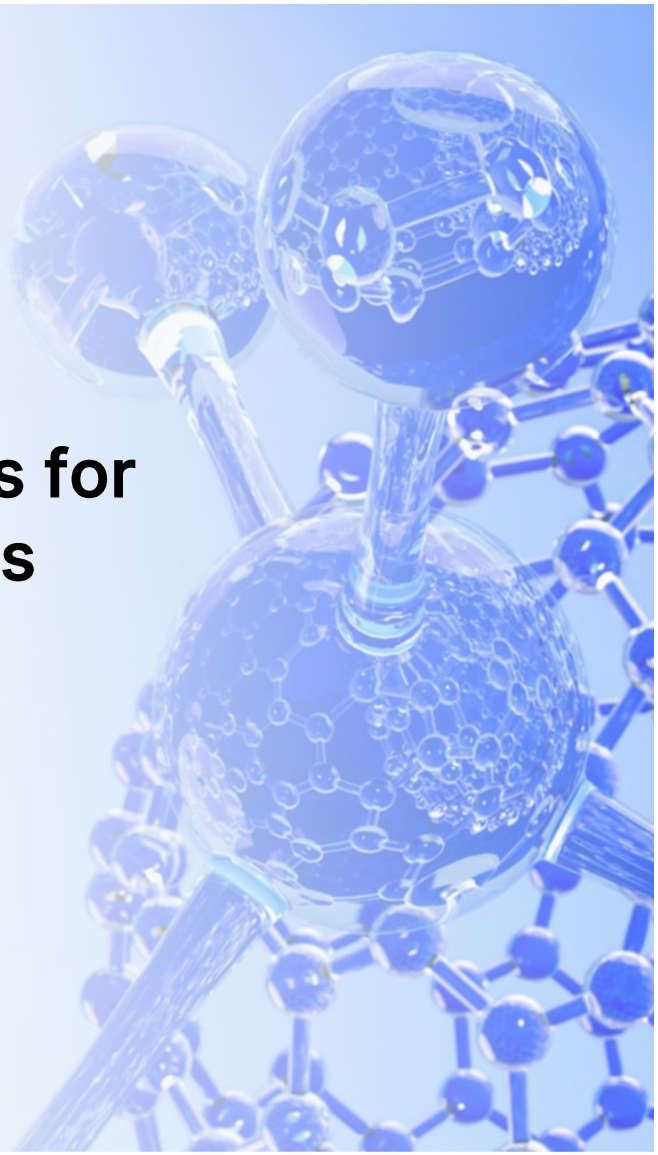
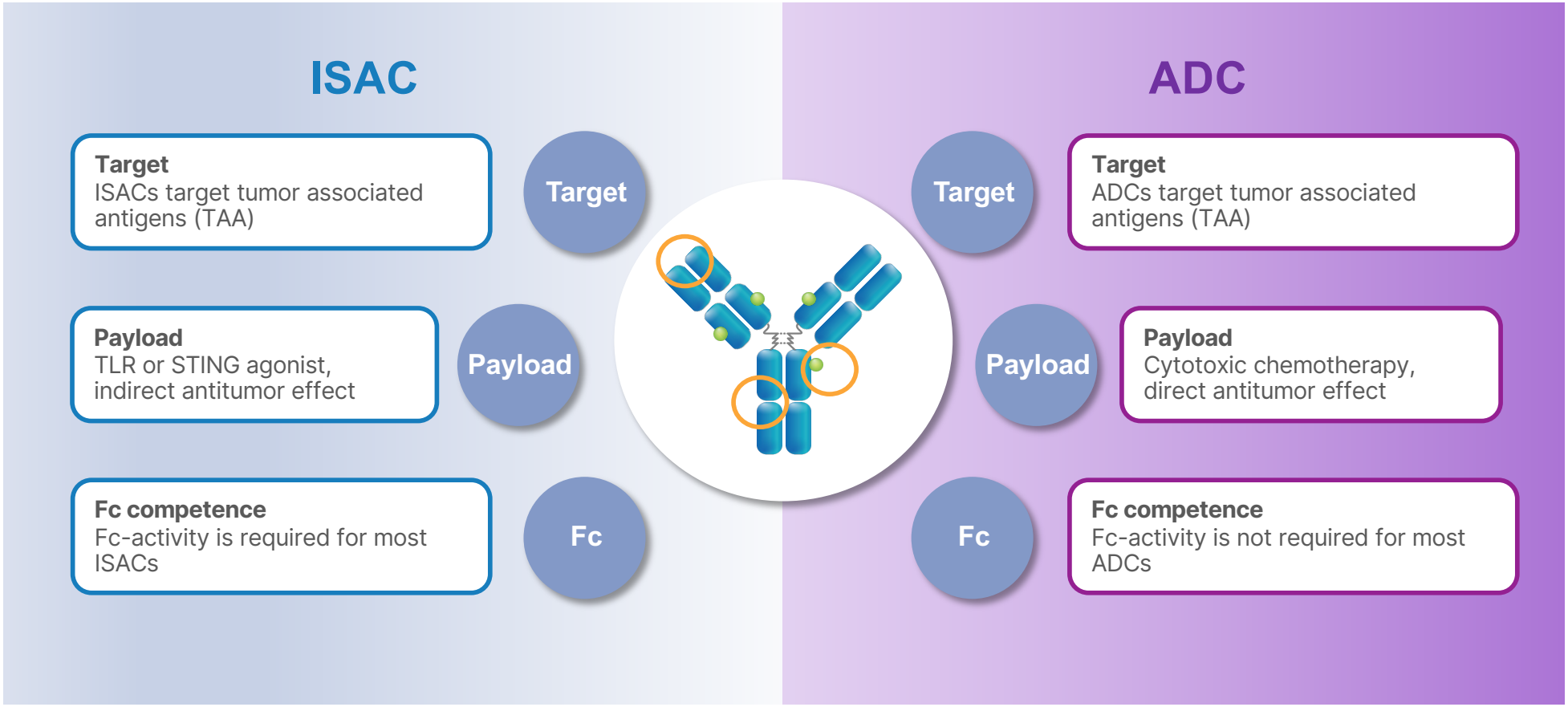


Immune-stimulating antibody conjugates (ISACs) are antibody drug conjugates (ADCs) that utilize immunostimulatory payloads

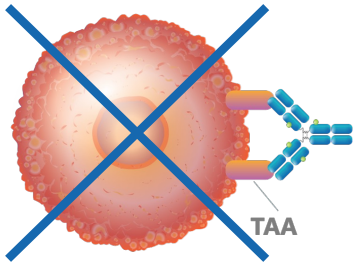


Considerations for toll-like receptor 7 (TLR7) agonists as ISAC payloads: localization and agonist structure

Localization

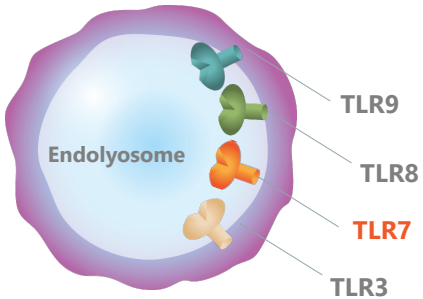
TLR7 is located in immune cells, not the tumor cells that ISACs typically target

TLR7 is not expressed in most tumor cells



Tumor Cell

TLR7 is found in the endolysosome of immune cells



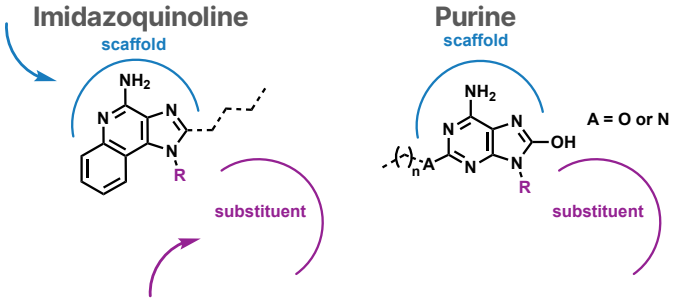
Immune cells, including pDCs

Agonist Structure

Substituent domain provides opportunity for payload optimization and linkability

Scaffold

- Occupies core binding domain
- Low chemical diversity tolerated



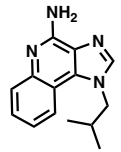
Substituent

- Occupies solvent exposed protein-protein interface
- Significant chemical diversity tolerated
- Allows for conjugable functional groups

pDCs = plasmacytoid dendritic cells

Despite efforts to tailor TLR7-agonist chemistry to the route of administration, cytokine-release syndrome (CRS) remains a major obstacle

Topical

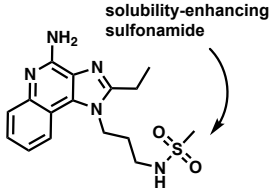


Imiquimod (R-837)

solubility: low
EC_{50}: µM
FIH 1989

Approved (1997)

Systemic

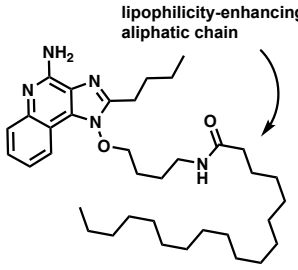


852a

solubility: 40x imiquimod
EC_{50}: nM
FIH 2007

Poorly tolerated

Intratumoral

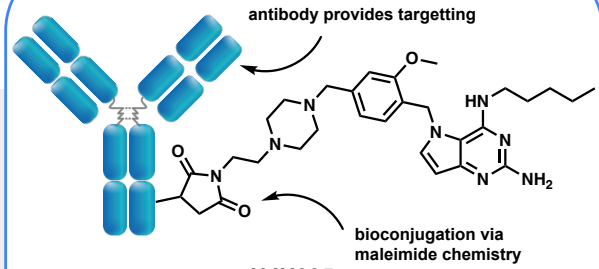


MEDI9197

solubility: very low
EC_{50}: nM
FIH 2015

Poorly tolerated

Systemic: ISAC



NJH395

solubility: very high
EC_{50}: pM
FIH 2019

Poorly tolerated

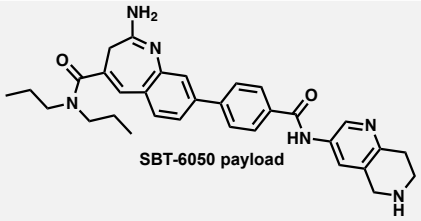
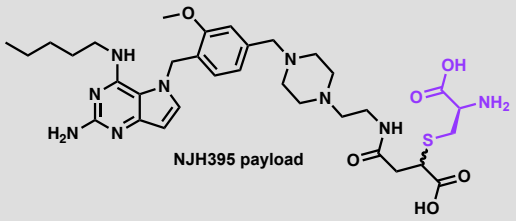
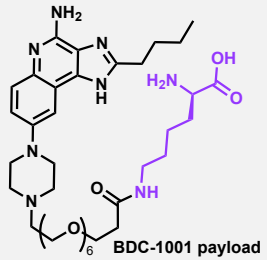
TLR7 potency

- Cytokine-release syndrome and other immune toxicities hamper the development of systemic and intratumorally administered TLR7 agonists

FIH = first-in-human

Poor tolerability and lack of efficacy are the biggest barriers to success for ISACs in the clinic

Dose limiting CRS → Low efficacy

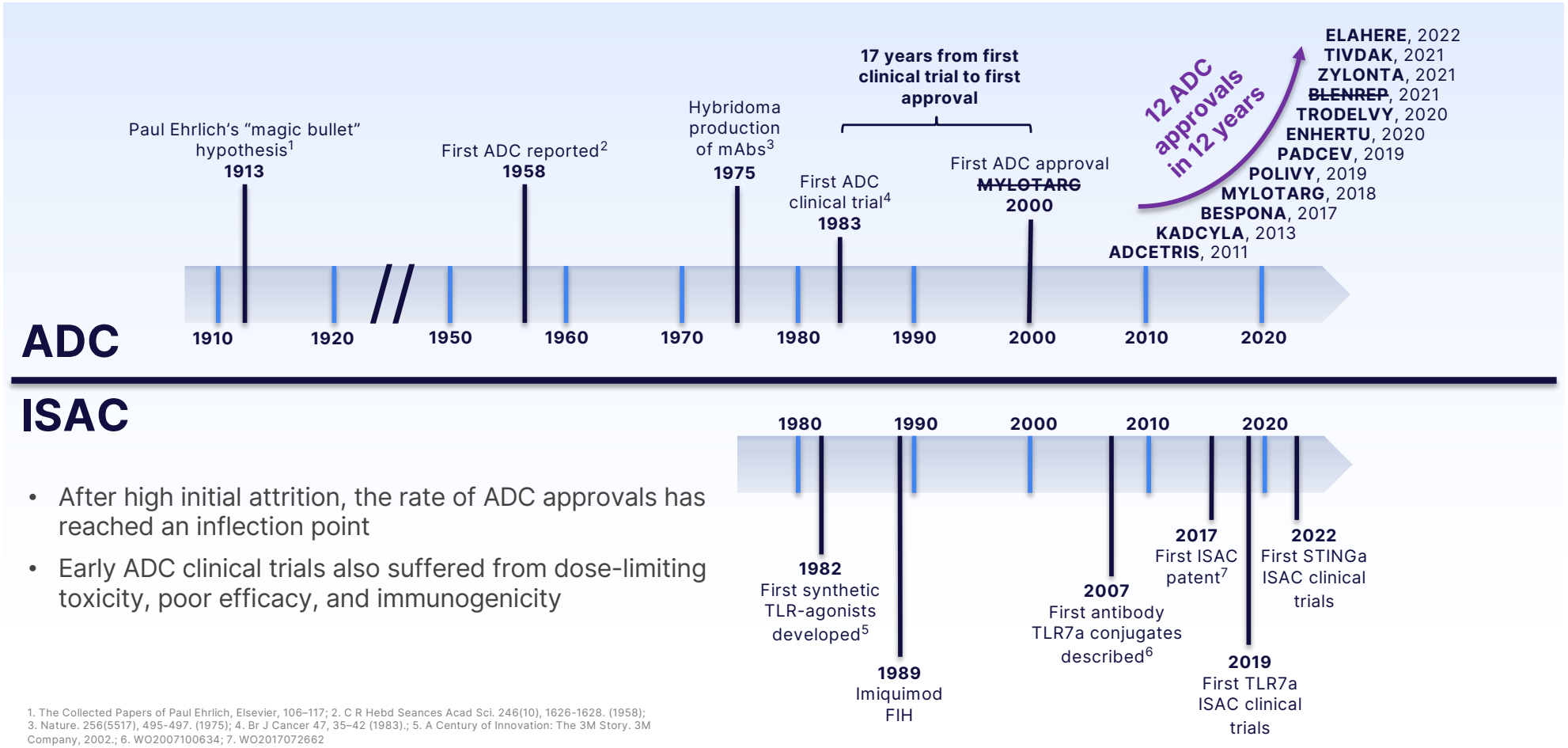
	Payload	ISAC Properties	Clinical Toxicities	Best Response
Silverback Therapeutics SBT-6050 **Anti-HER2** (pert epitope)		Benzazepine **TLR8a** cleavable, DAR8, cysteine conjugate	**Dosed up to 1.2 mg/kg q2w** **CRS** in 4/32 pts; **G3** hypotension in 6/32 pts; 1 **G5** in combo with Pembro	**PR** 1/18; **SD** 14/18 **Trial halted**
Novartis NJH395 **Anti-HER2** (tras epitope)		Pyrrolopyrimidine **TLR7a** noncleavable DAR4 SS cysteine conjugate	**Dosed up to 1.6 mg/kg** **Single dose** **G2** CRS in 10/18 of pts; **ADAs** in 14/14 pts tested	**SD** 9/14 **Trial halted**
Bolt Biotherapeutics BDC1001 **Anti-HER2** (tras epitope)		Imidazoquinoline **TLR7/8a** noncleavable DAR2 lysine conjugate	**Dosed up to 20 mg/kg q3w** **No DLTs** observed to date; **MTD** has not been reached **No CRS** **No ADAs**	**PR** 1/40; **SD** 12/40 ***Trial ongoing***

CRS = cytokine-release syndrome
DAR = drug-to-antibody ratio
SS = site specific

SD = stable disease
PR = partial response
ADAs = antidrug antibodies

G# = grade # adverse event
pts = patients

ISACs: a newcomer in cancer immunotherapy compared to cytotoxic ADCs

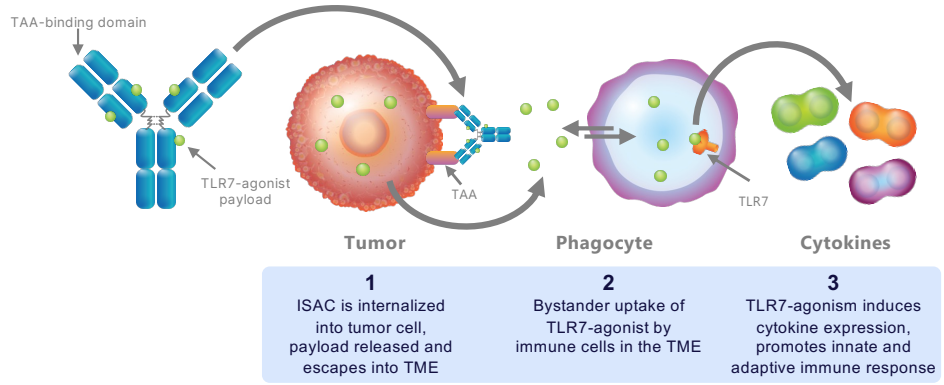


- After high initial attrition, the rate of ADC approvals has reached an inflection point
- Early ADC clinical trials also suffered from dose-limiting toxicity, poor efficacy, and immunogenicity

1. The Collected Papers of Paul Ehrlich, Elsevier, 106–117; 2. C R Hebd Seances Acad Sci. 246(10), 1626-1628. (1958); 3. Nature. 256(5517), 495-497. (1975); 4. Br J Cancer 47, 35–42 (1983).; 5. A Century of Innovation: The 3M Story. 3M Company, 2002.; 6. WO2007100634; 7. WO2017072662

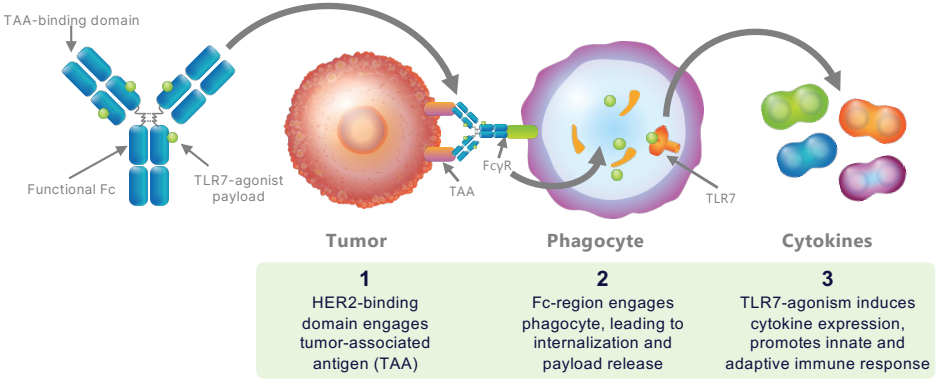
Distinct mechanisms-of-action have been proposed to describe the activity of ISACs

Proposed MOA 1: Bystander effect



As initially reported by:
Novartis

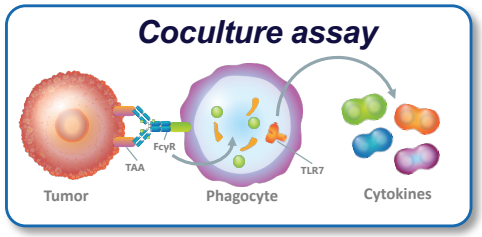
Proposed MOA 2: Immune-engagement



As initially reported by:
Silverback Therapeutics &
Bolt Biotherapeutics

TME = tumor microenvironment

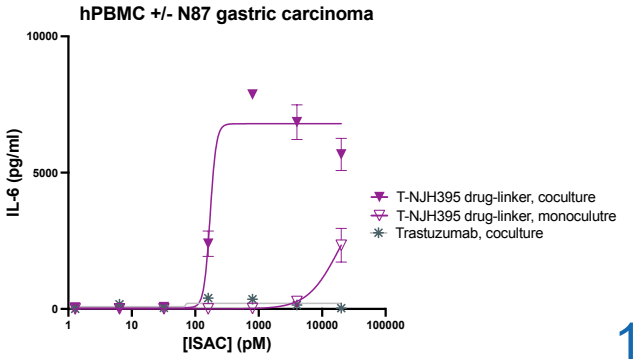
Four independent observations support the immune-engagement MOA



Benchmark ISAC

- Trastuzumab
- NJH395 drug-linker
- Stochastic DAR = 4
- Cysteine conjugation

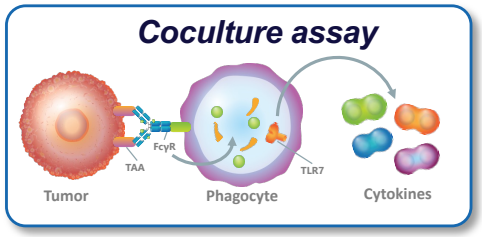
Coculture assays reveal ISAC activity in vitro



1 2
3 4

hPBMCs = human blood peripheral mononuclear cells

Four independent observations support the immune-engagement MOA



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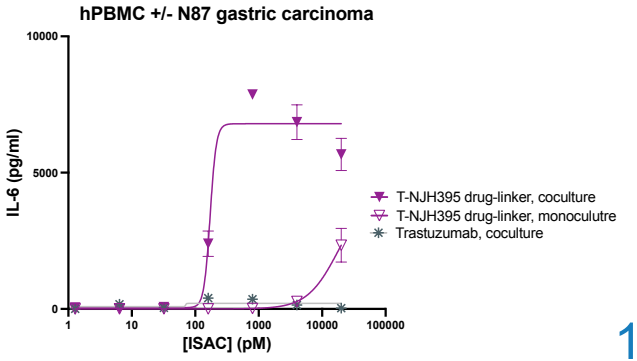
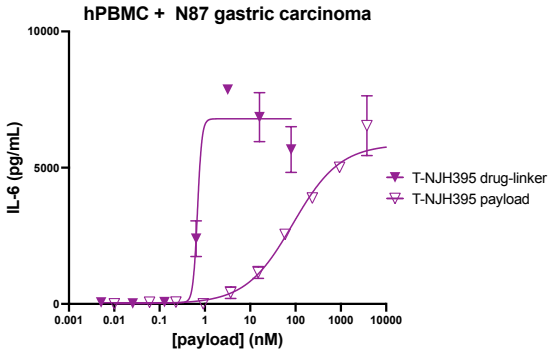


ISACs are >100x more potent than free payload



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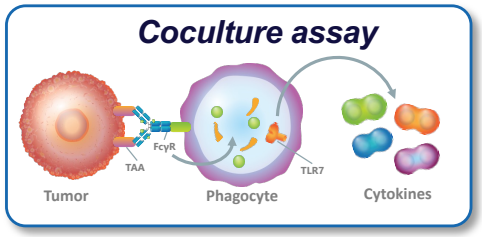

Trastuzumab NJH395 drug-linker

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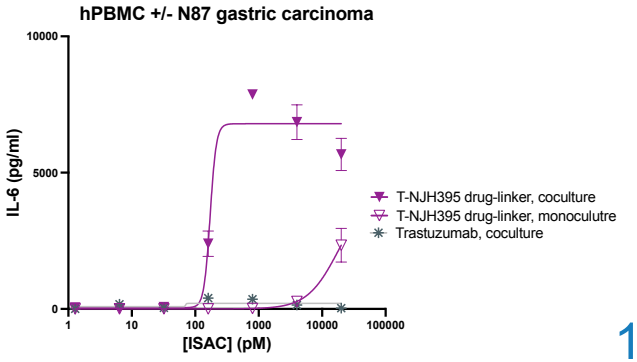

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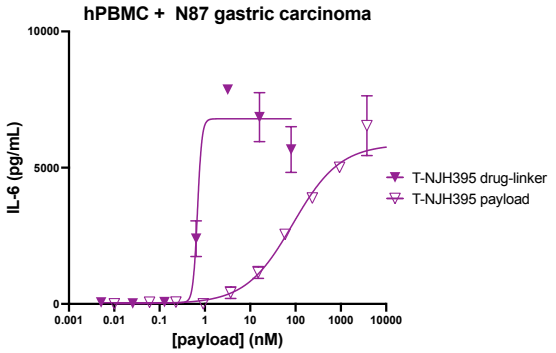
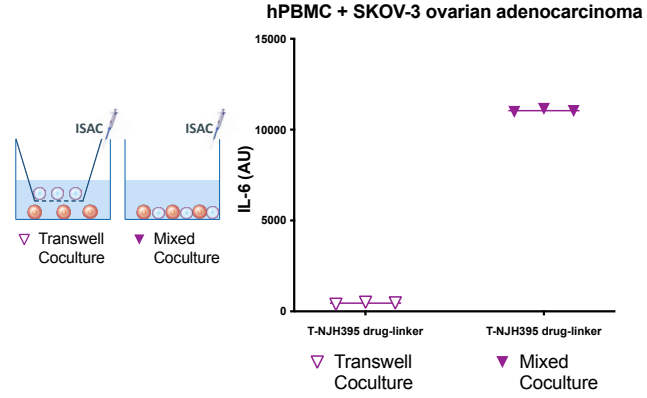


Cell/cell contact is important



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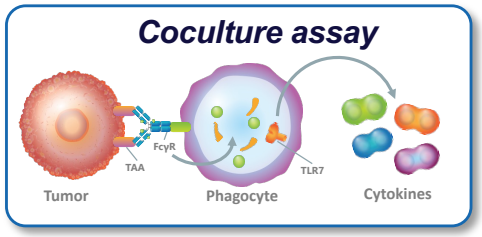

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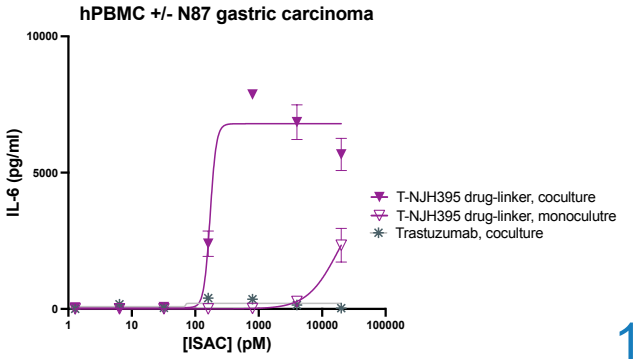

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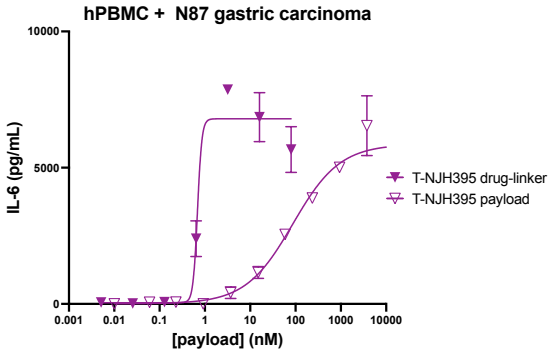
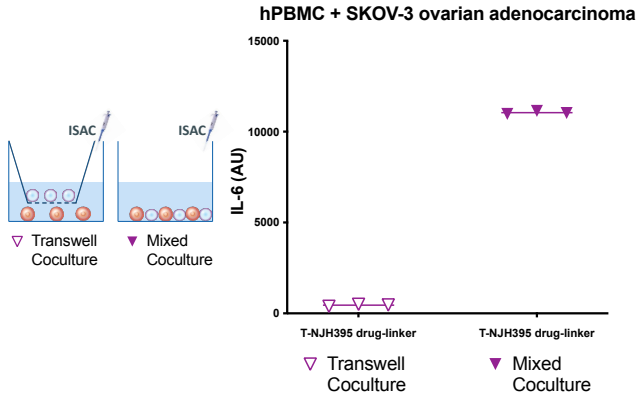
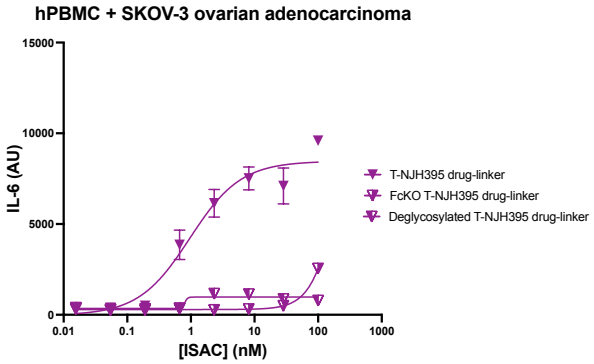


3 Cell/cell contact is important



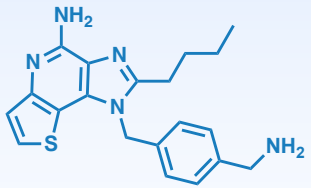
4 Fc-knockout inhibits activity



hPBMCs = human blood peripheral mononuclear cells

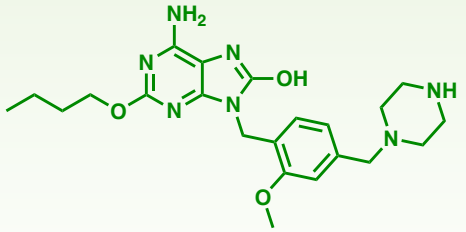
Zymeworks has evaluated two scaffold classes as ISAC payloads

Imidazothienopyridine



- Dual TLR7/8-agonist
- Structural analog to the imidazoquinoline

Purine

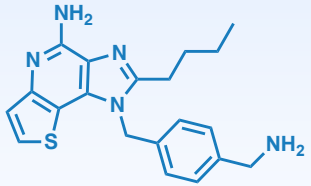


- Pure TLR7 agonist
- Broadly explored as small molecule agonist

- Trastuzumab was used as a model system to compare our drug-linkers to clinical benchmarks

Zymeworks has evaluated two scaffold classes as ISAC payloads

Imidazothienopyridine



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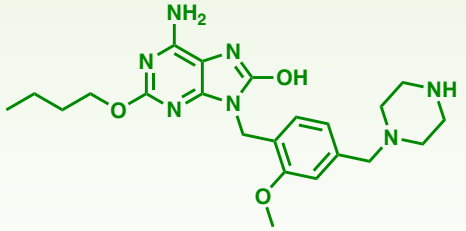
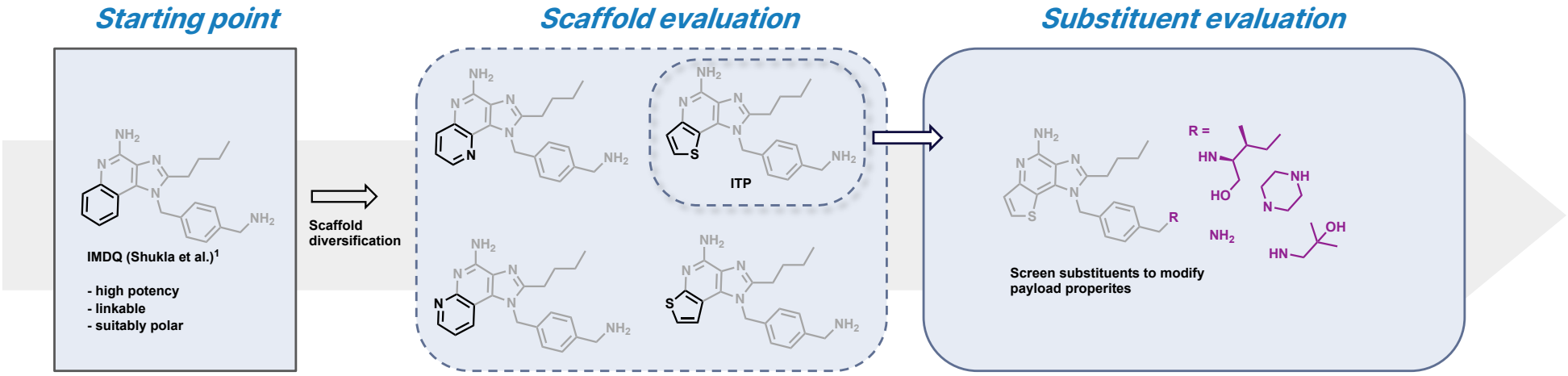


- Pure TLR7 agonist
- Broadly explored as small molecule agonist

- Trastuzumab was used as a model system to compare our drug-linkers to clinical benchmarks

New imidazoquinoline analogs were prepared and evaluated as ISAC payloads



1. Identify a core scaffold with robust activity
2. Optimize the substituent portion for use as a bioconjugate

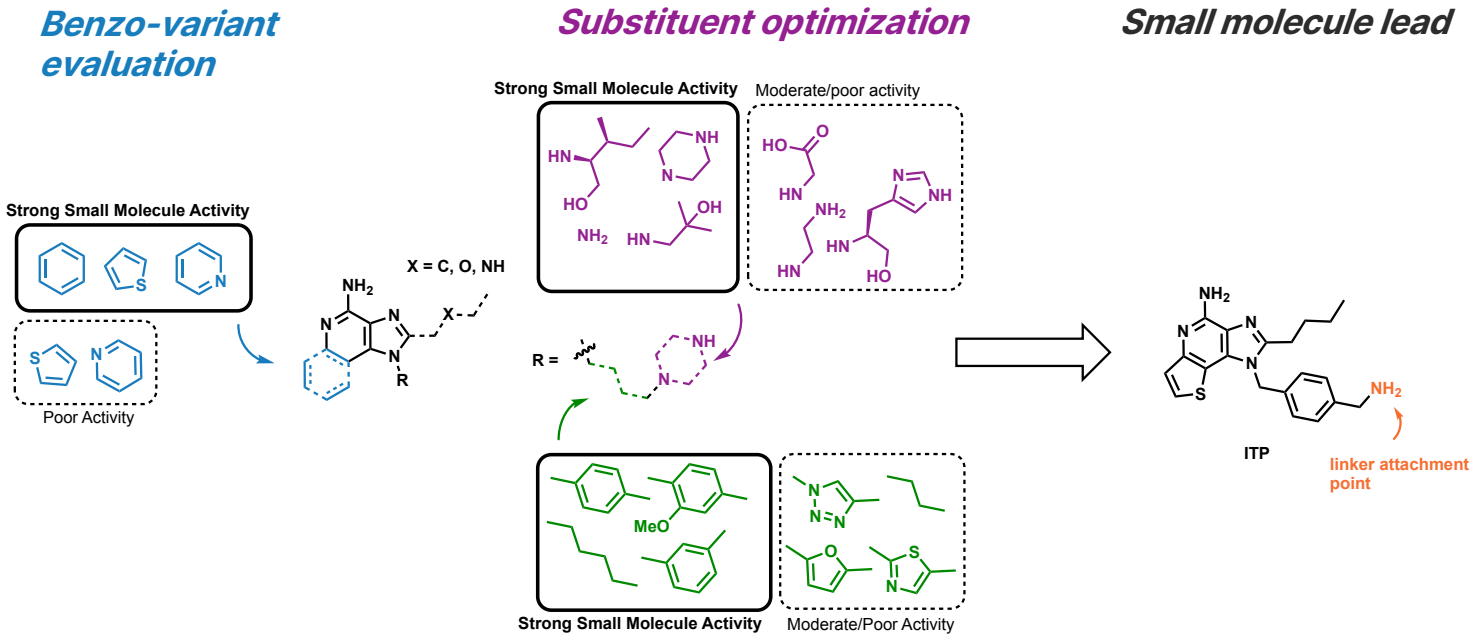
manuscript submitted

1. Bioorg Med Chem 6386 (2010) Lett. 20(22):6384-

Structure-activity relationship (SAR) trends for activity of imidazoquinoline-type small molecules

SAR learnings:

1. **Benzo-variants** are active as ADC payloads
2. **Benzyl and MeO-Benzyl** spacers are preferred, enhance murine activity
3. **Meta-orientation** spacers have higher TLR8 activity
4. **Mono and diamine substituents** are active and provide linkable handle



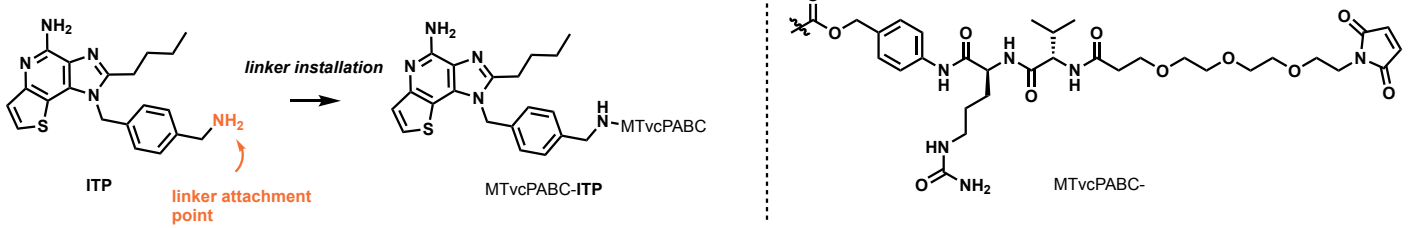
manuscript submitted

Novel imidazoquinoline-type drug-linkers generate trastuzumab-ISACs with favorable biophysical characteristics

Drug-linkers were synthesized from novel payloads

- Cleavable linker system

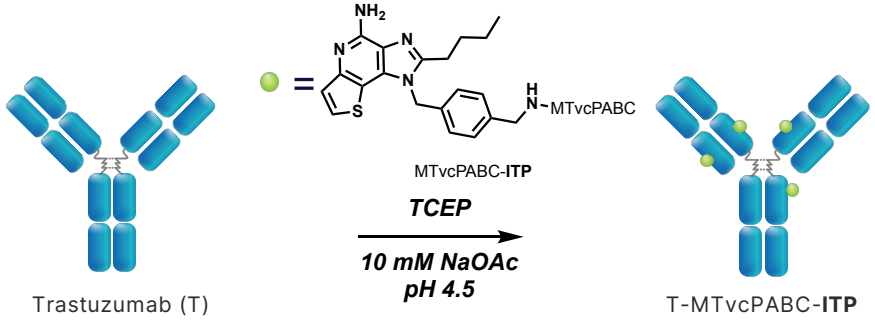
Drug-linker generation



ISACs were generated

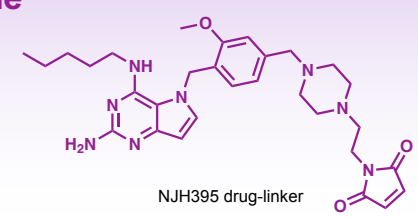
- Trastuzumab
- Cysteine conjugation
- Stochastic DAR = 4

ISAC generation



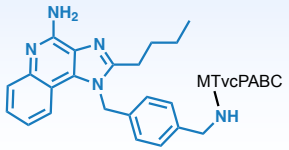
In a murine in vitro assay, comparable activity was observed for imidazoquinoline and pyrrolopyrimidine ISACs

Pyrrolopyrimidine (benchmark)

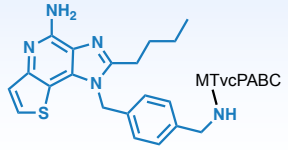


NJH395 drug-linker

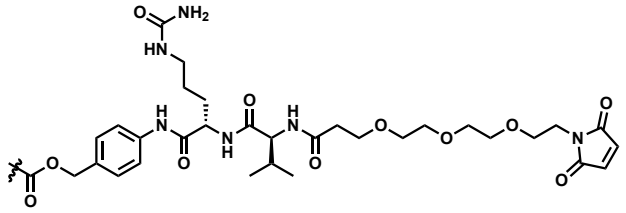
Imidazoquinoline



MTvcPABC-**IMDQ**

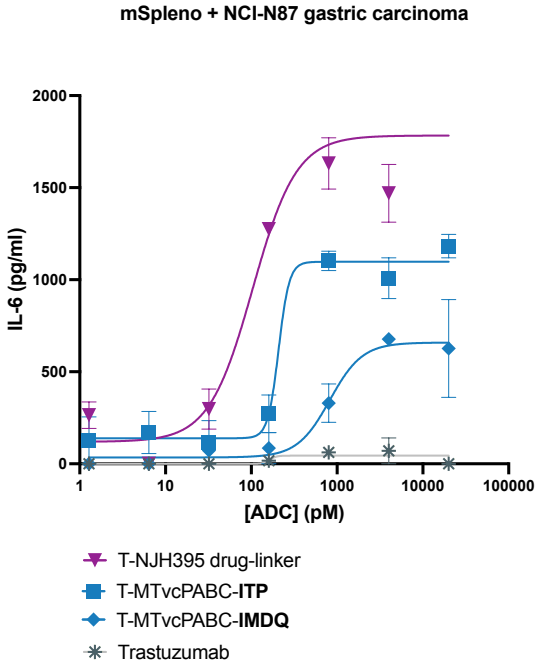


MTvcPABC-**ITP**



MTvcPABC-

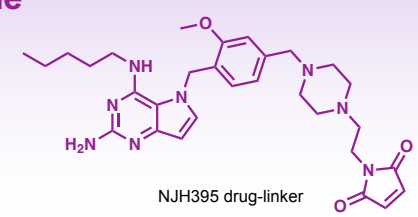
mSpleno = murine splenocytes



	Murine activity	Human activity
Pyrrolopyrimidine	✅	
Imidazoquinoline	✅	

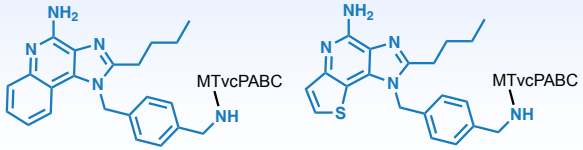
In a murine CDX model, comparable activity was observed from imidazoquinoline and pyrrolopyrimidine ISACs

Pyrrolopyrimidine (benchmark)



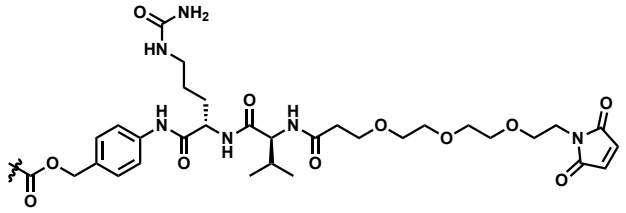
NJH395 drug-linker

Imidazoquinoline



MTvcPABC-**IMDQ**

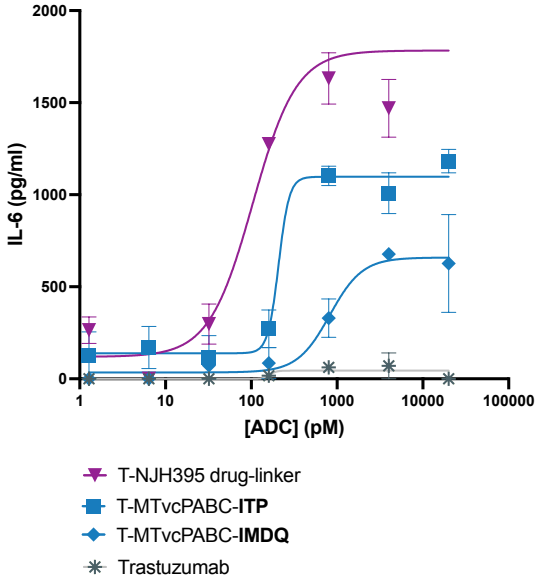
MTvcPABC-**ITP**



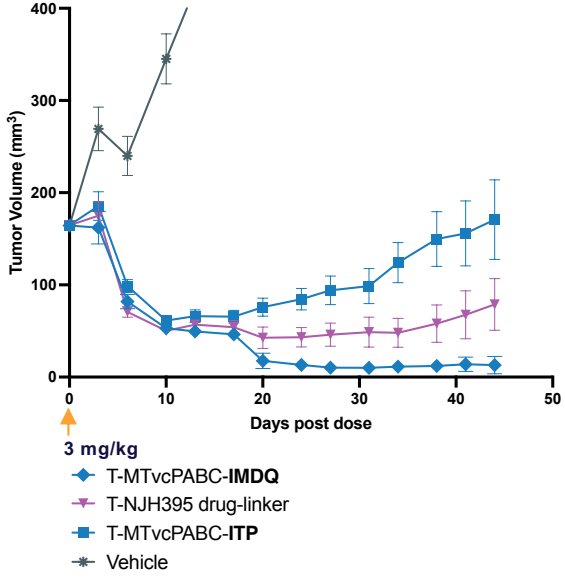
MTvcPABC-

mSpleno = murine splenocytes

mSpleno + NCI-N87 gastric carcinoma



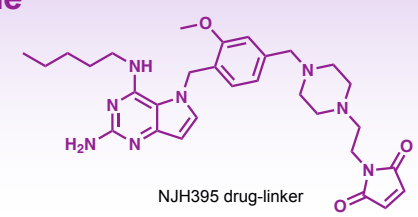
NCI-N87 gastric carcinoma
HER2-high
BALB/c nude



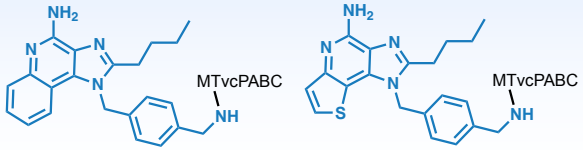
	Murine activity	Human activity
Pyrrolopyrimidine	✅	
Imidazoquinoline	✅	

Purine ISACs showed consistent and robust responses across in vitro assays with human and murine primary cells

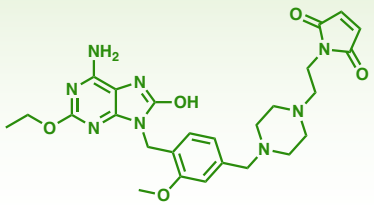
Pyrrolopyrimidine (benchmark)

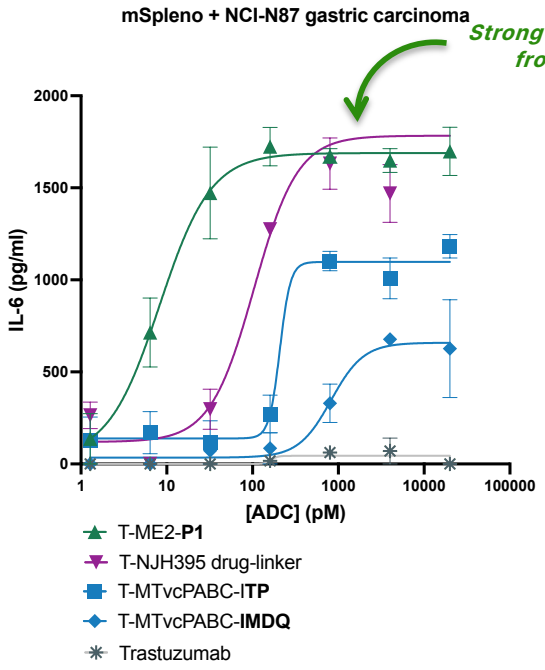


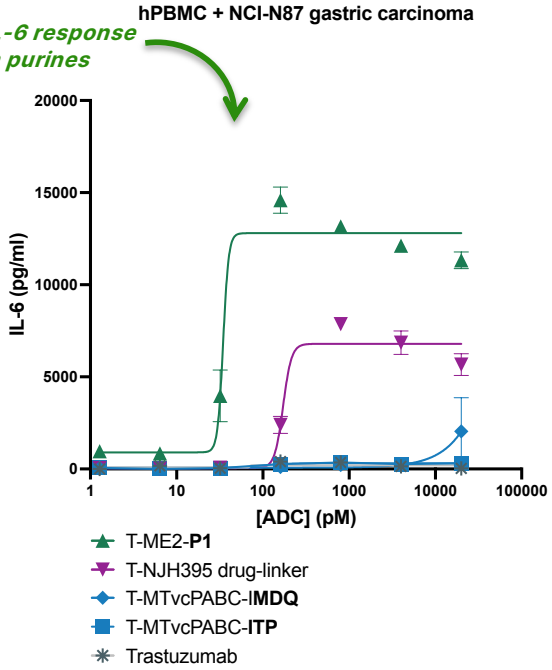
Imidazoquinoline



Purine





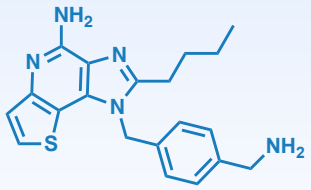


	Murine activity	Human activity
Pyrrolopyrimidine	✅	✅
Imidazoquinoline	✅	?
Purine	✅	✅

mSpleno = murine splenocytes
hPBMCs = human blood peripheral mononuclear cells

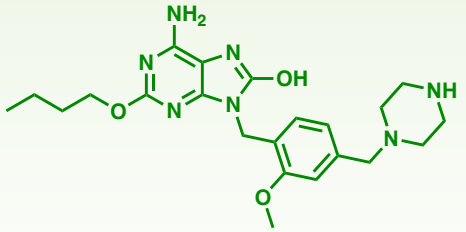
Zymeworks has evaluated two scaffold classes as ISAC payloads

Imidazothienopyridine



- Dual TLR7/8-agonist
- Structural analog to the imidazoquinoline

Purine

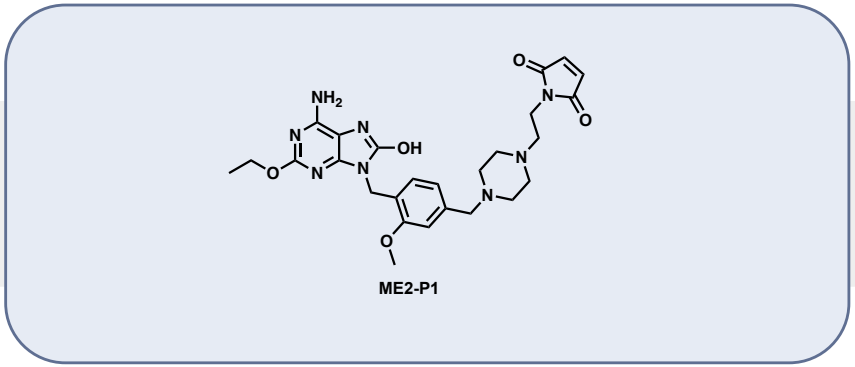


- Pure TLR7 agonist
- Broadly explored as small molecule agonist

- Trastuzumab was used as a model system to compare our drug-linkers to clinical benchmarks

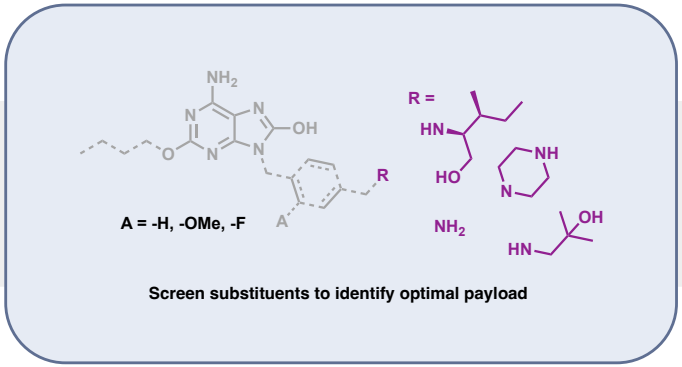
A purine drug-linker platform was selected for further development

Scaffold validation



1. Identify scaffolds with strong species cross-reactivity

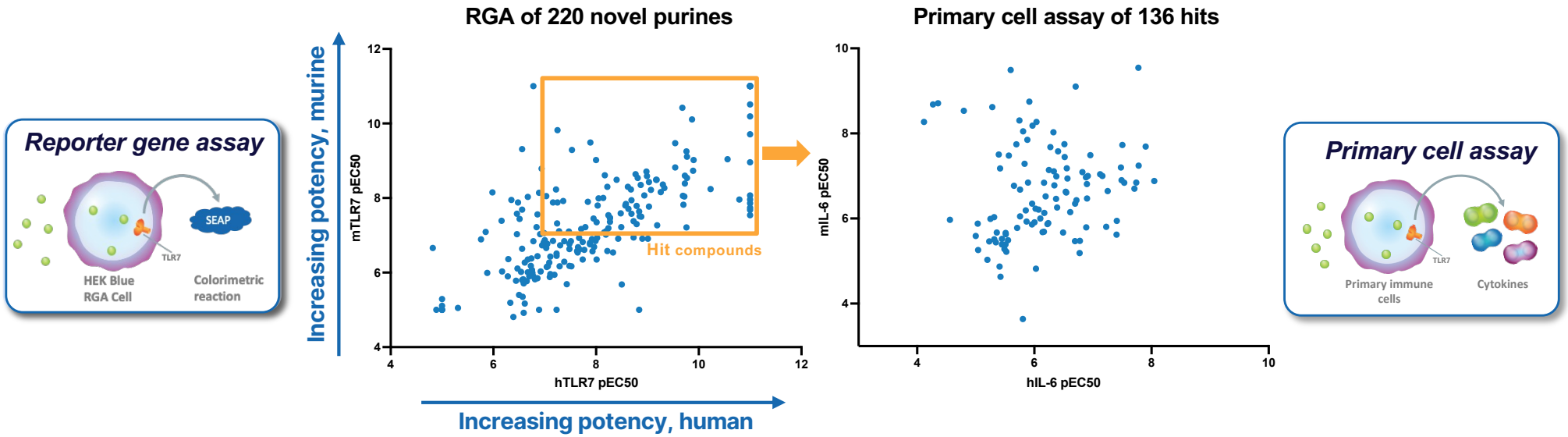
Substituent evaluation



2. Optimize the substituent portion for use as a bioconjugate

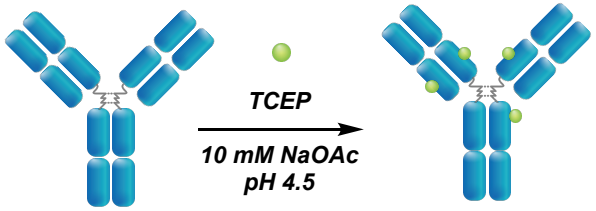
manuscript in preparation

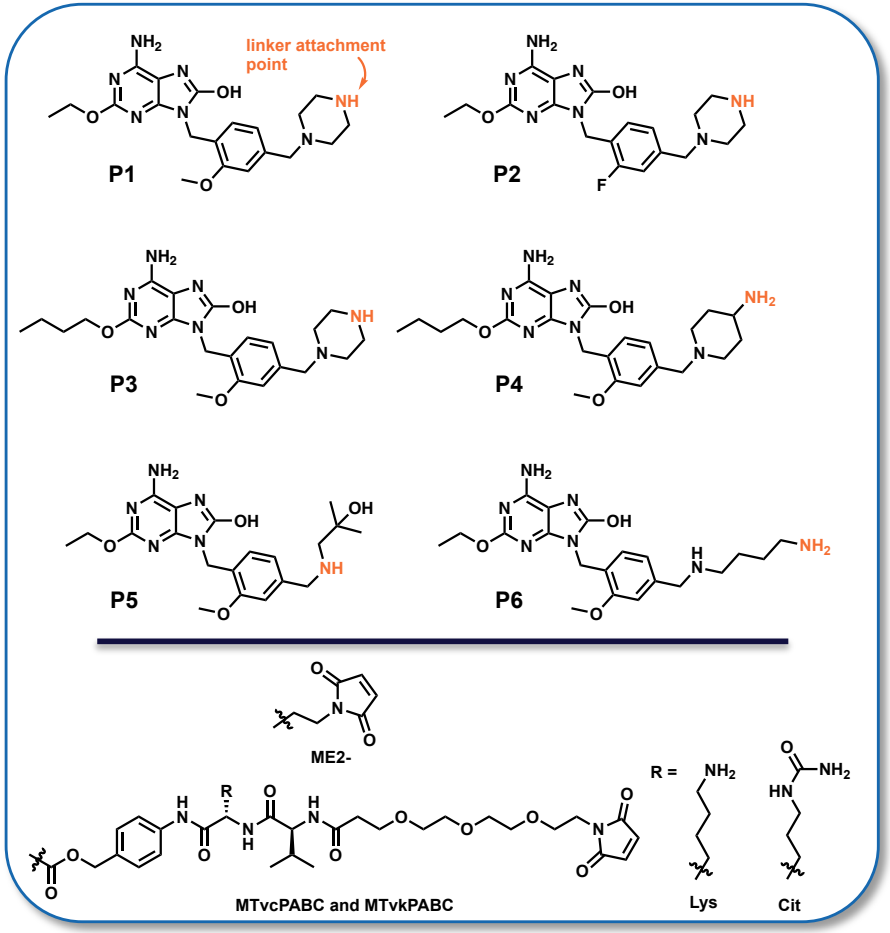
Small molecules were screened and selected for ISAC conjugation based on potency and structural diversity



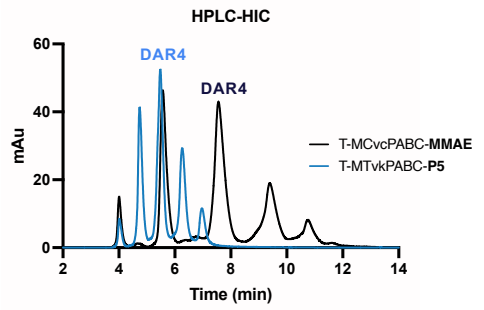
- 220 novel small molecule purines were synthesized
- Hit compounds (pEC50 > 7 in both assays) were evaluated against human and murine primary immune cells
- Drug-linkers were generated from the 40 most promising compounds

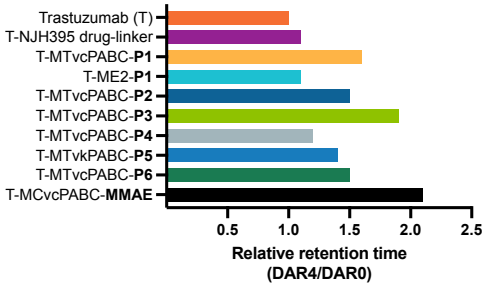
Novel purine drug-linkers generate trastuzumab-ISACs with favorable biophysical characteristics





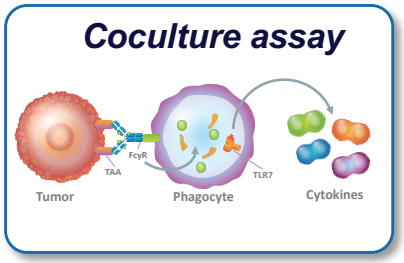
- Purine drug-linkers were conjugated to trastuzumab to achieve an average drug-to-antibody ratio (DAR) of 4

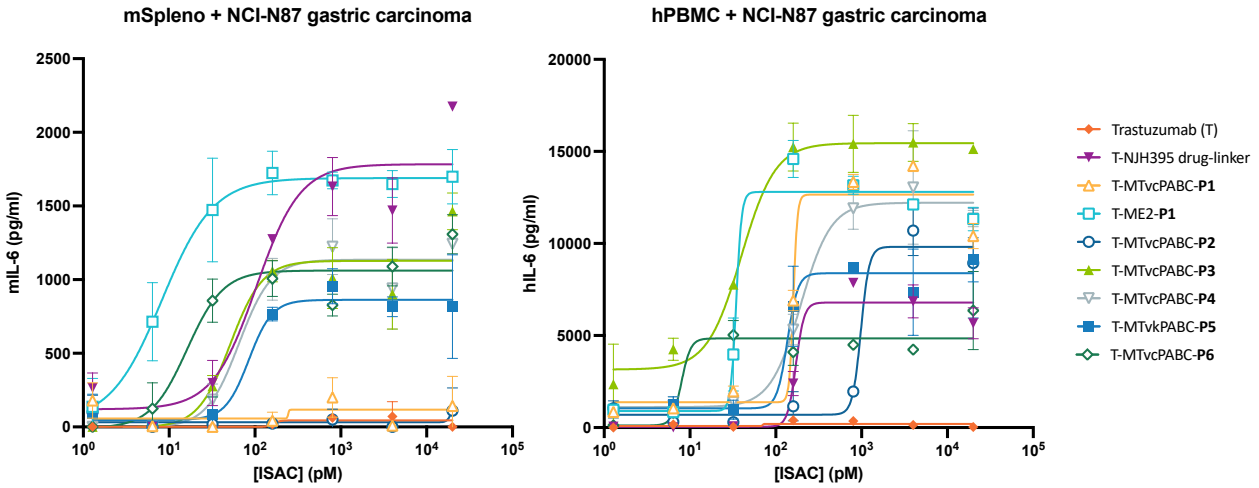




- The resulting ISACs demonstrated low aggregation and acceptable hydrophobicity

Purine ISACs drive potent immune response in both mouse and human coculture systems





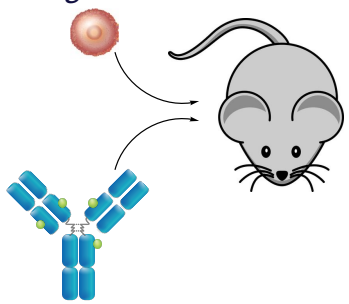
- Human PBMCs or murine splenocytes were cocultured with N87 tumor cells in the presence of indicated ISACs.
- ISACs capable of inducing high levels of IL-6 from cocultures of tumor cells and primary immune cells were selected for in vivo studies

mSpleno = murine splenocytes
hPBMCs = human blood peripheral mononuclear cells

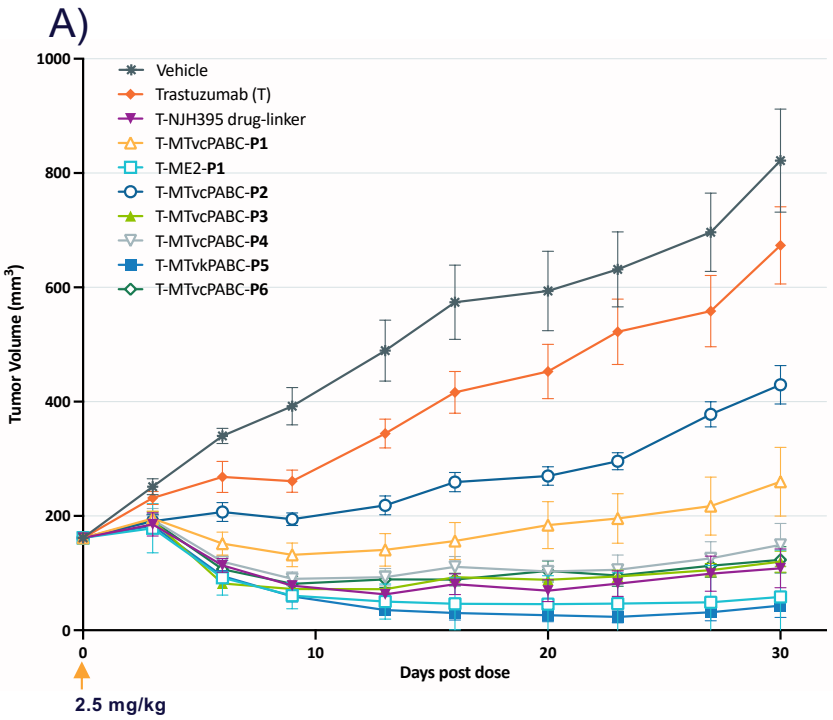
Novel purine ISACs show similar in vivo efficacy to T-NJH395 benchmark

In vivo cell-derived xenograft (CDX)

Engraft tumor



Administer ISAC at 2.5 mg/kg



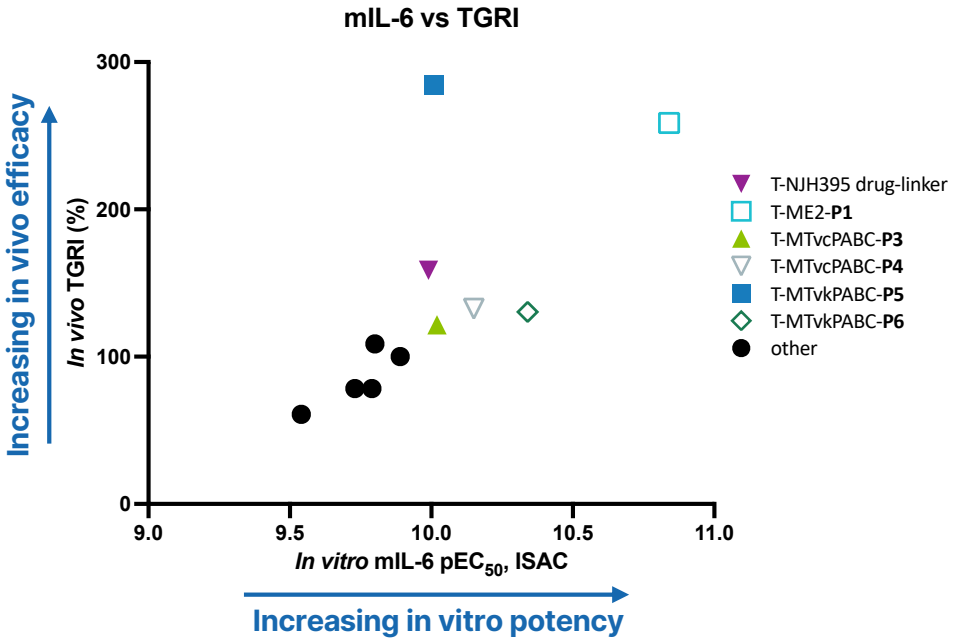
Antitumor activity of purine-based TLR7 agonists conjugated to trastuzumab (DAR = 4) in an NCI-N87 tumor cell-line derived xenograft BALB/c nude model.

In vivo tumor growth rate inhibition (TGRI) correlates with in vitro IL-6 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
- In vivo tumor growth rate inhibition (TGRI) was calculated according to the following formula:

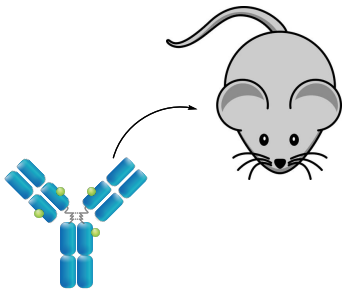
$$TGRI = [1 - \frac{tumor\ growth\ rate\ kinetic\ of\ treated\ group}{tumor\ growth\ rate\ kinetic\ of\ control\ group}]\ x\ 100$$

Tumor growth rate inhibition is correlated with mIL-6 induction from coculture assay



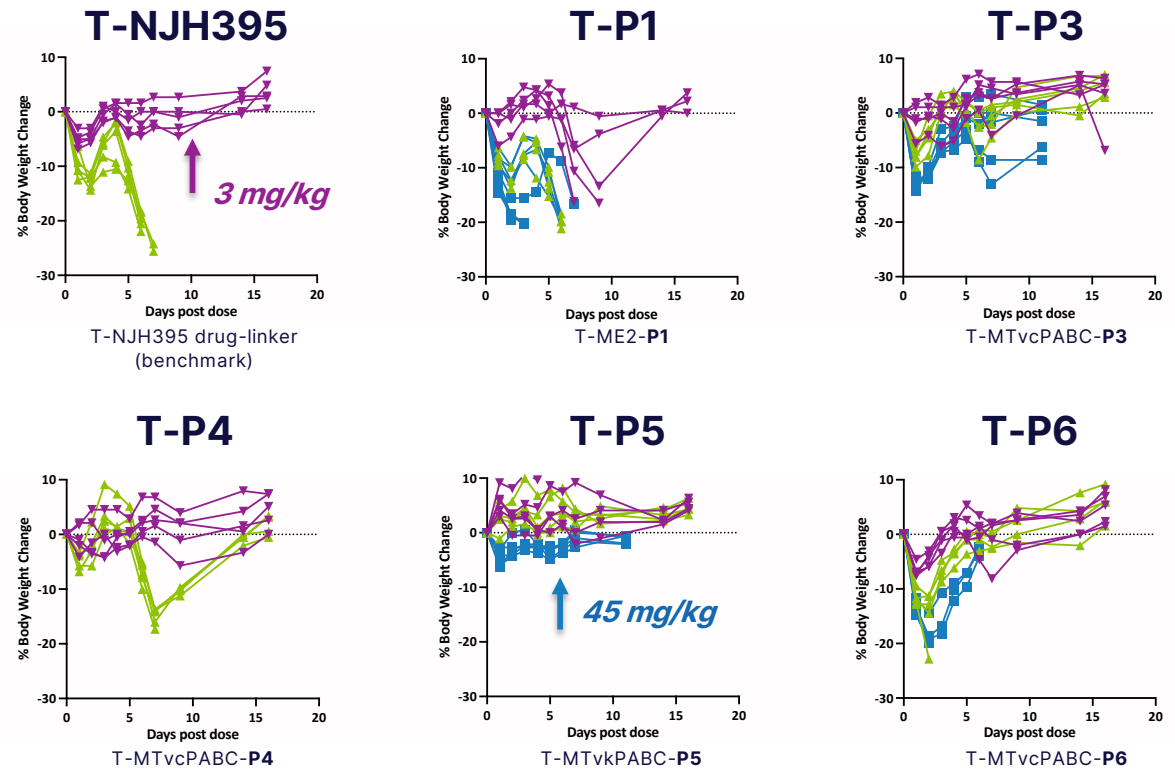
Novel purine ISACs show superior in vivo tolerability to T-NJH395 benchmark

Murine tolerability (BALB/c mice)



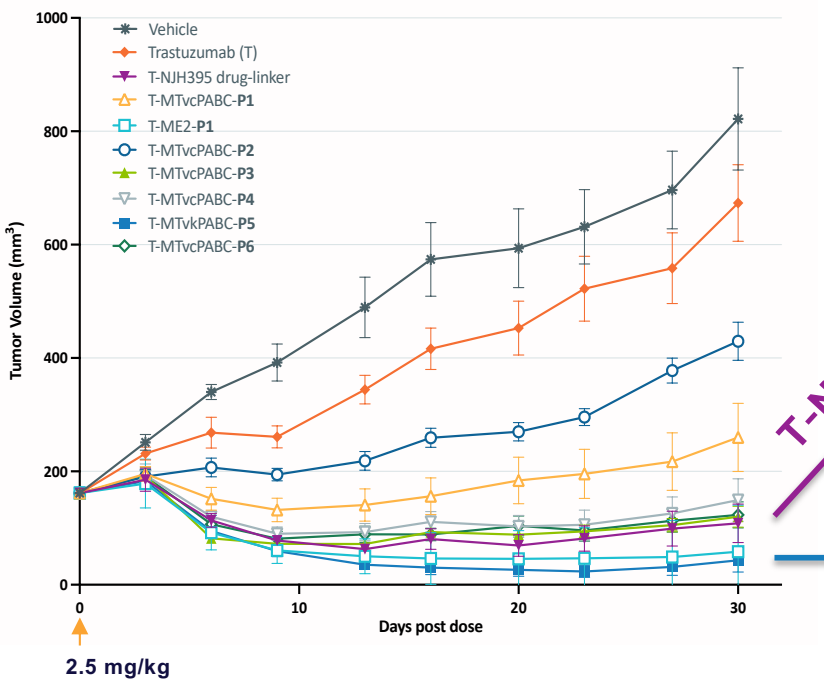
Administer ISAC up to 45 mg/kg

3 mg/kg
15 mg/kg
45 mg/kg



Body weight change over time of healthy, immunocompetent BALB/c mice following single intravenous administration of 3, 15, or 45 mg/kg of the respective ISACs.

Head-to-head comparison highlights therapeutic benefit of purine drug-linkers

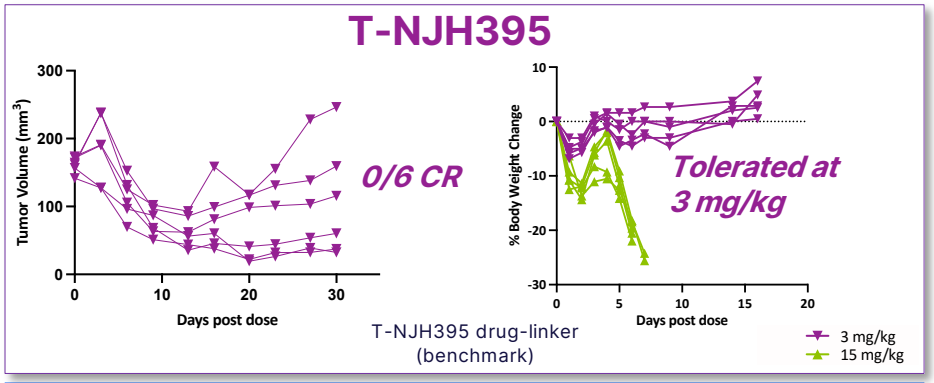


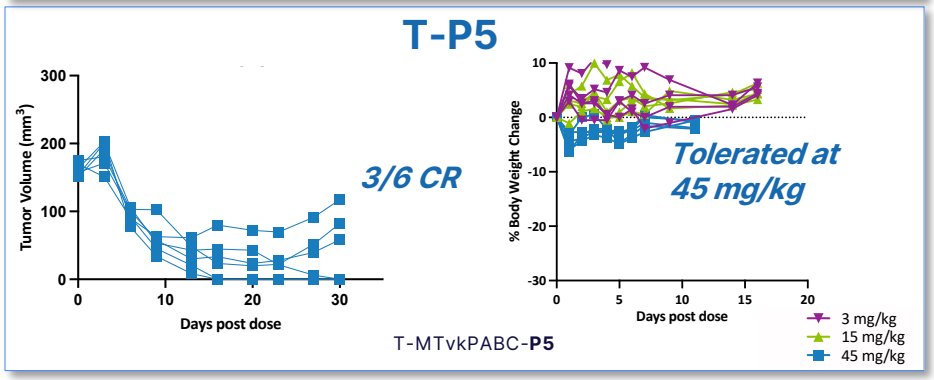
T-NJH395

T-P5

Efficacy

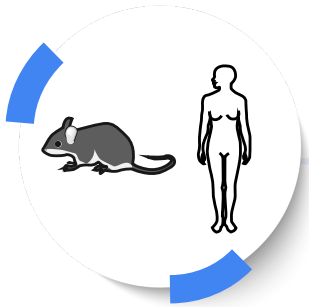
Tolerability





- T-MTvkPABC-**P5** exhibits superior tolerability while maintaining the efficacy of T-NJH395 drug-linker

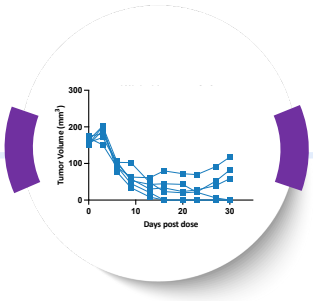
Purine-based ISACs have demonstrated compelling preclinical activity



Cross-species Activity

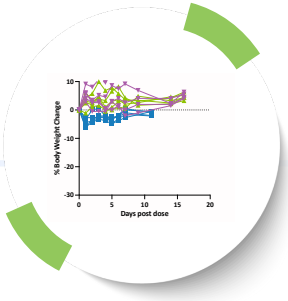
Purine-based ISACs showed **strong activity on both murine and human immune cells** in vitro.

This cross-species conservation of activity negates the use of surrogate molecules for in vivo studies, providing greater translational relevance than other platforms



Efficacy

In vivo efficacy studies in an N87 xenograft model indicate **activity comparable or superior to the clinically evaluated NJH395 drug-linker**



Tolerability

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 technology
Contact Lucas Donigian, Executive Director, Business Development at lucas.donigian@zymeworks.com

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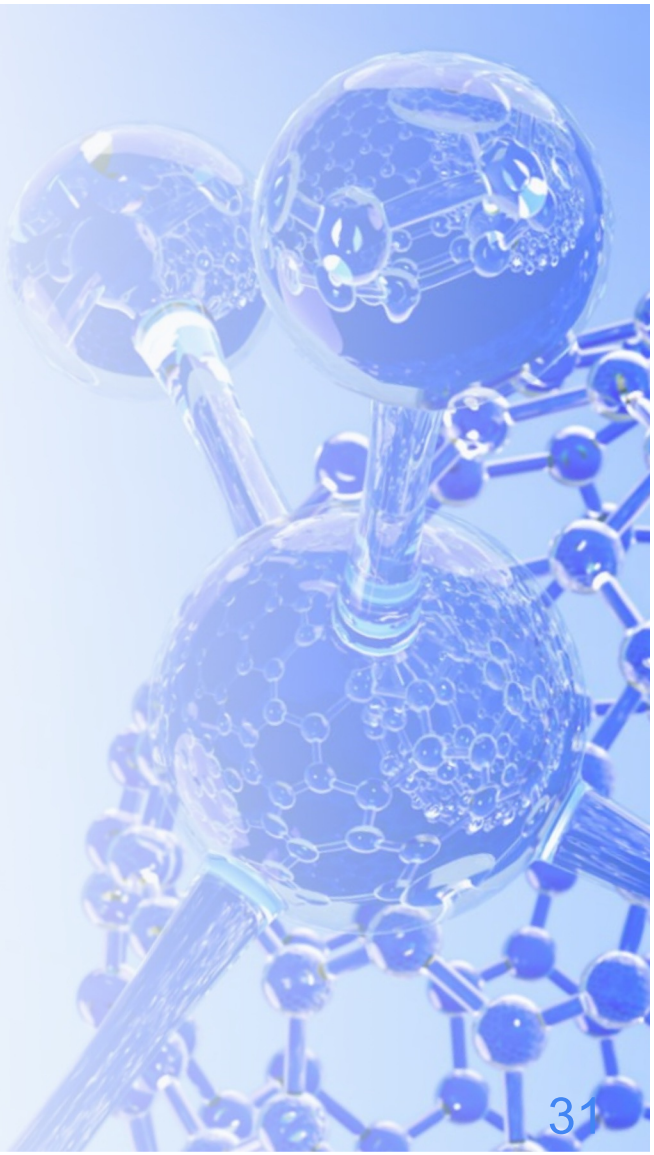
- Cathy Dang

Thank You



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