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

Graham Garnett
Scientist, Medicinal Chemistry, ADC Therapeutic Development
Zymeworks BC Inc.

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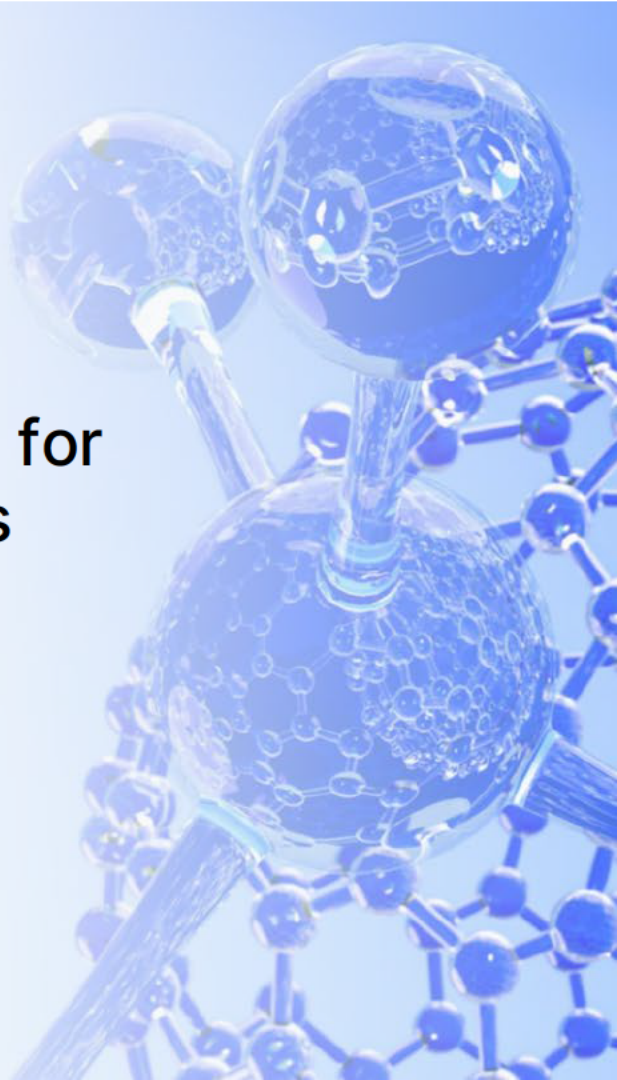

# Immune-stimulating Antibody Conjugates (ISACs) are Antibody-drug Conjugates (ADCs) that Utilize Immunostimulatory Payloads

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## ISAC

**Target**
ISACs target tumor associated antigens (TAA)

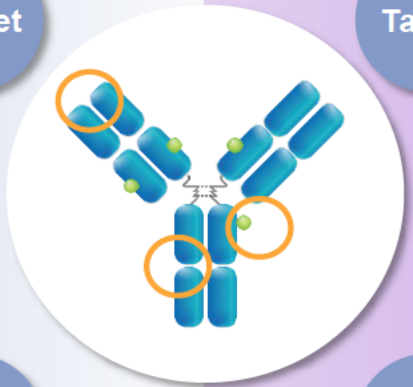
Target

**Payload**
TLR or STING agonist, indirect antitumor effect

Payload

**Fc competence**
Fc-activity is required for most ISACs

Fc



## ADC

Target

**Target**
ADCs target tumor associated antigens (TAA)

Payload

**Payload**
Cytotoxic chemotherapy, direct antitumor effect

Fc

**Fc competence**
Fc-activity is not required for most ADCs

# Considerations for Toll-like Receptor 7 (TLR7) Agonists as ISAC Payloads: Localization and Agonist Structure

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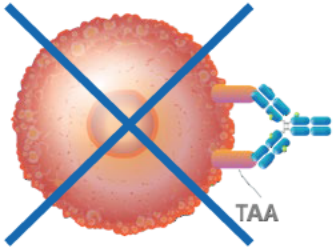
## Localization

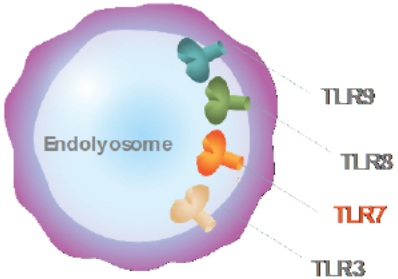
TLR7 is located in immune cells, not the tumor cells that ISACs typically target

TLR7 is not expressed in most tumor cells




Tumor Cell

TLR7 is found in the endolysosome of immune cells



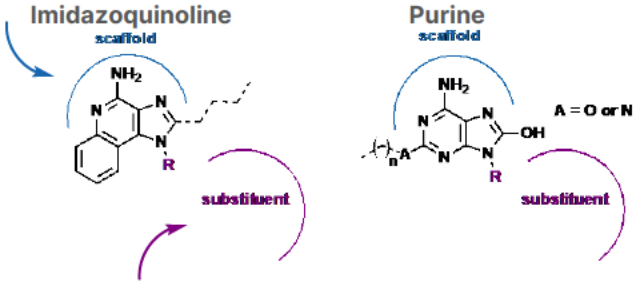

Immune cells, including pDCs

## 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

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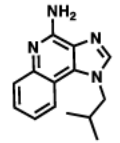
## Topical

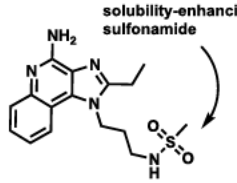


**Imiquimod (R-837)**

solubility: low
$EC_{50}$: µM
FIH 1989

**Approved (1997)**

## Systemic

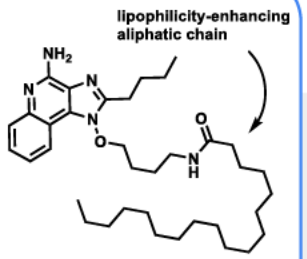



**852a**

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

**Poorly tolerated**

## Intratumoral

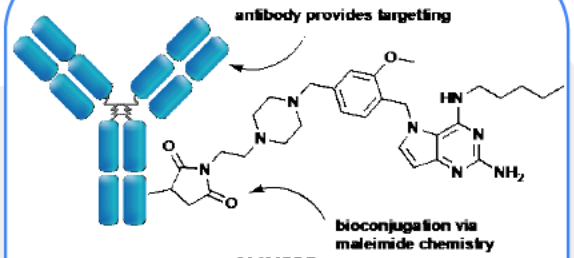



**MEDI9197**

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

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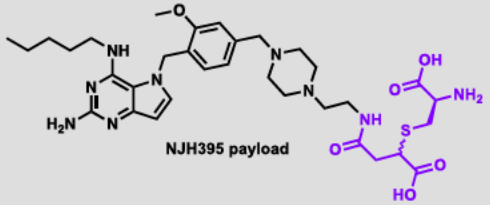
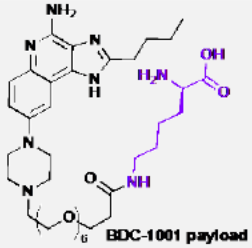
Dose limiting CRS → Low efficacy

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

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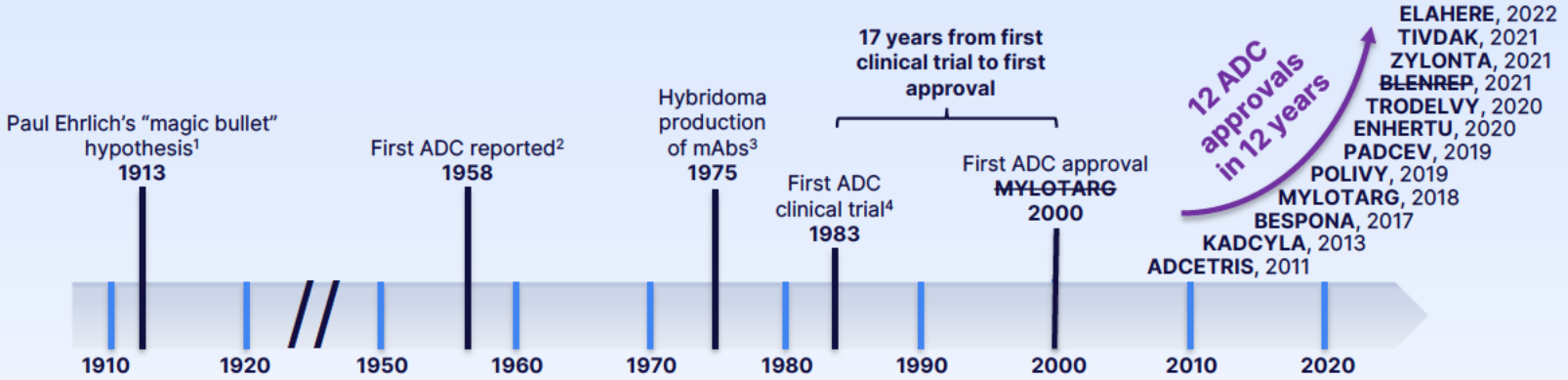
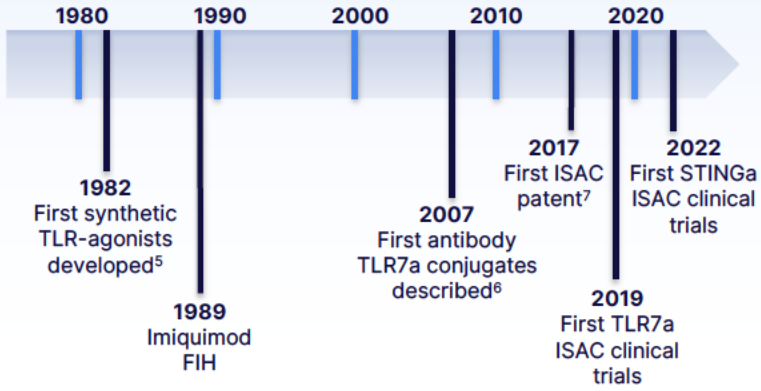



## ISAC

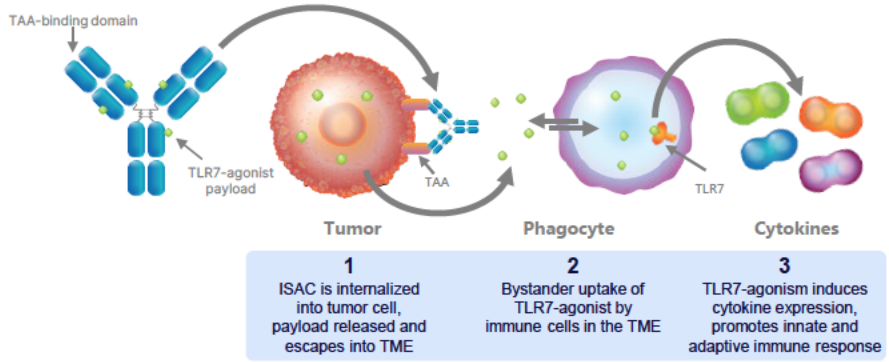
- After high initial attrition, the rate of ADC approvals has reached an inflection point
- Early ADC clinical trials also suffered from dose-limiting toxicity, poor efficacy, and immunogenicity




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

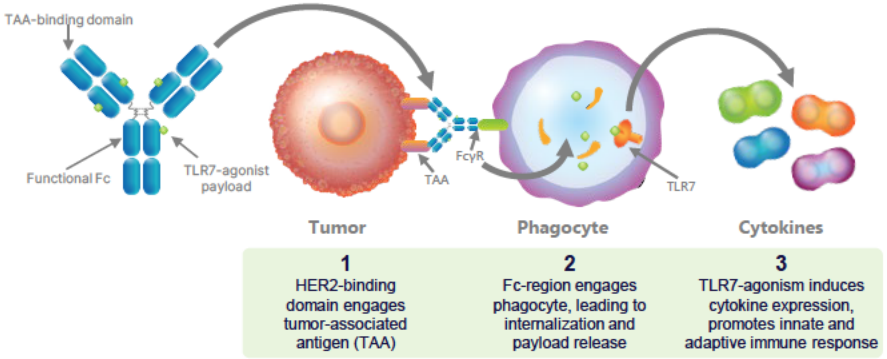
# Distinct Mechanisms-of-action have been Proposed to Describe the Activity of ISACs

## Proposed MOA 1: Bystander effect




**As initially reported by:**
Novartis

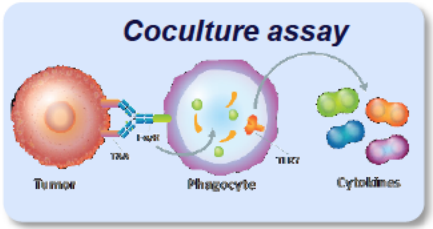
## Proposed MOA 2: Immune-engagement

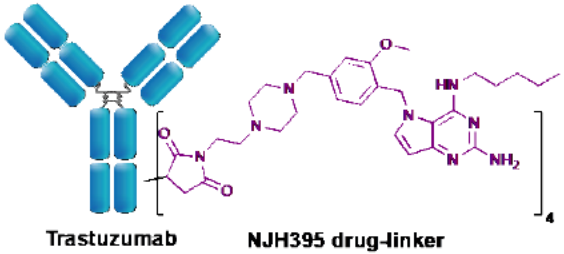



**As initially reported by:**
Silverback Therapeutics & Bolt Biotherapeutics

TME = tumor microenvironment

# Four Independent Observations Support the Immune-engagement MOA

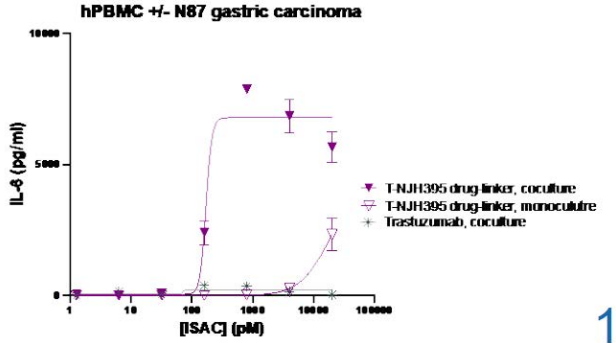



## Benchmark ISAC

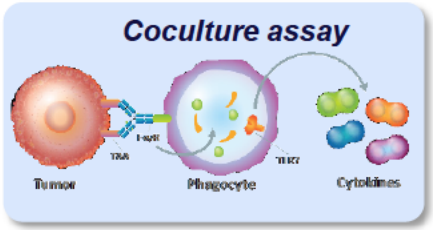
- Trastuzumab
- NJH395 drug-linker
- Stochastic DAR = 4
- Cysteine conjugation

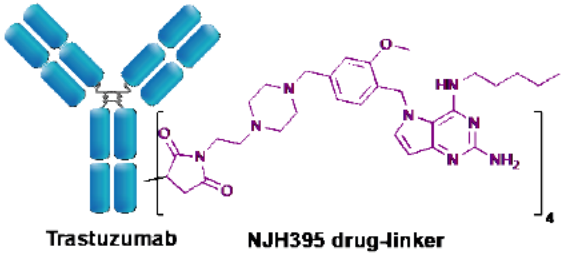
*Coculture assays reveal ISAC activity in vitro*




hPBMCs = human blood peripheral mononuclear cells

# Four Independent Observations Support the Immune-engagement MOA

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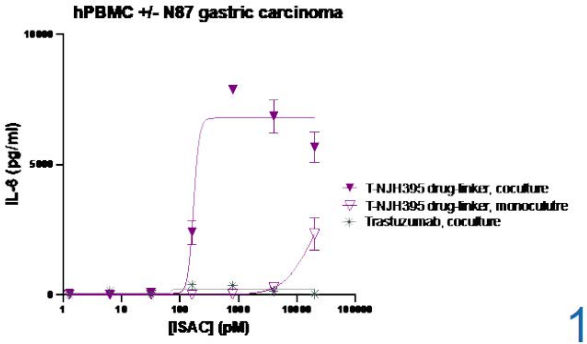
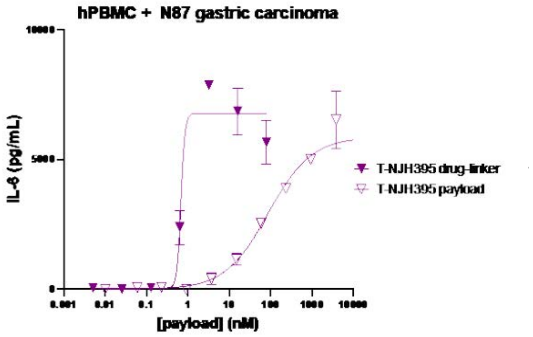



### *ISACs are >100x more potent than free payload*




1 2
3 4

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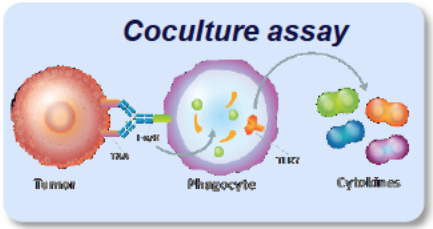


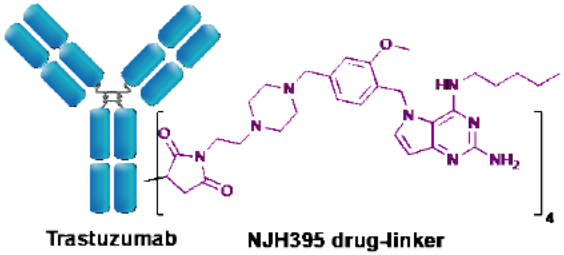


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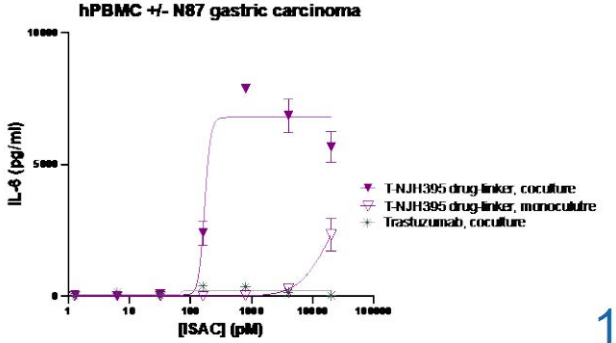


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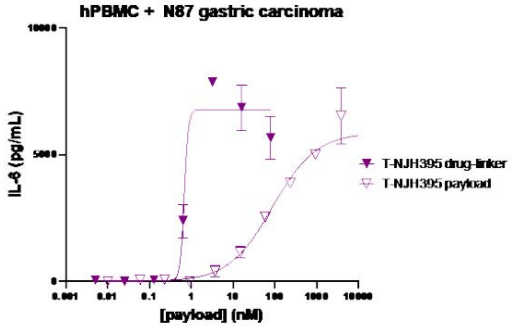
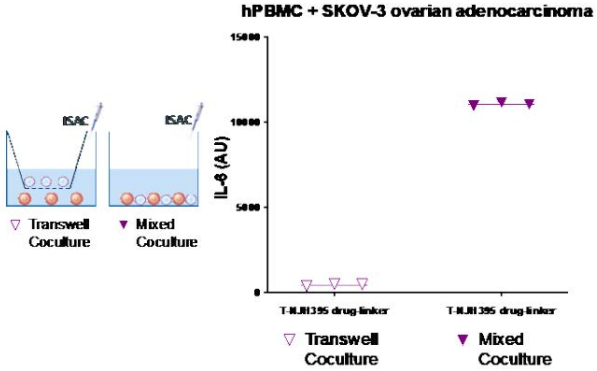



### Cell/cell contact is important

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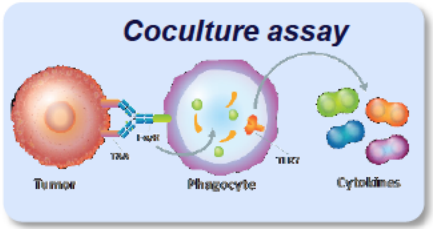


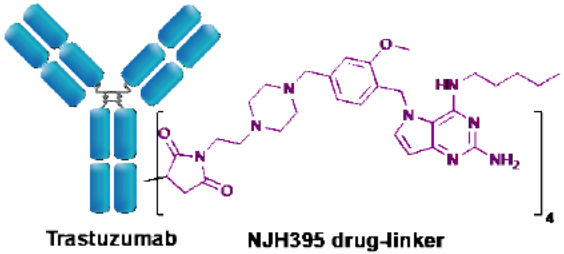


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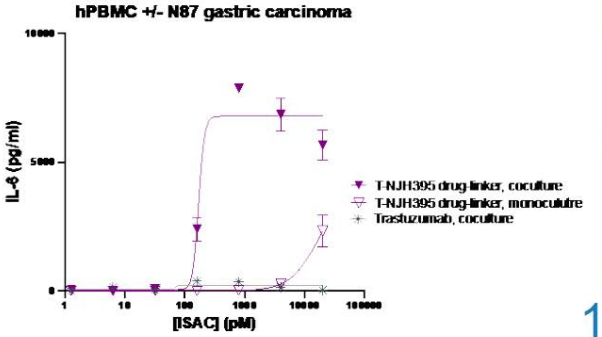
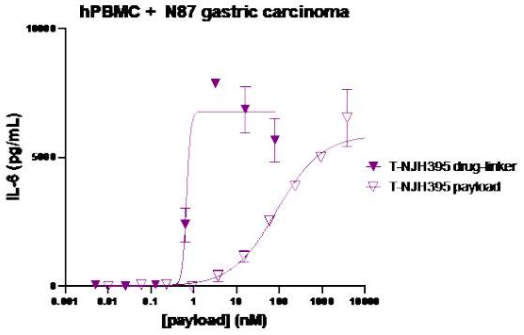



### 2 ISACs are >100x more potent than free payload

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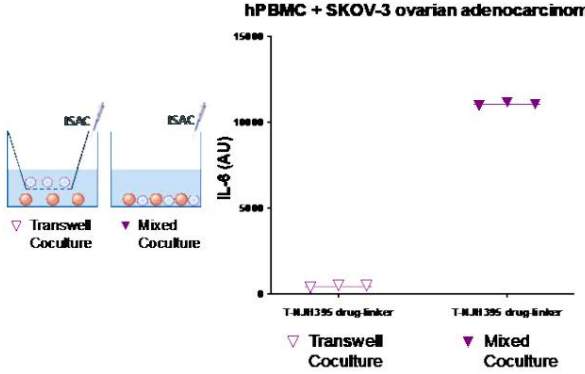
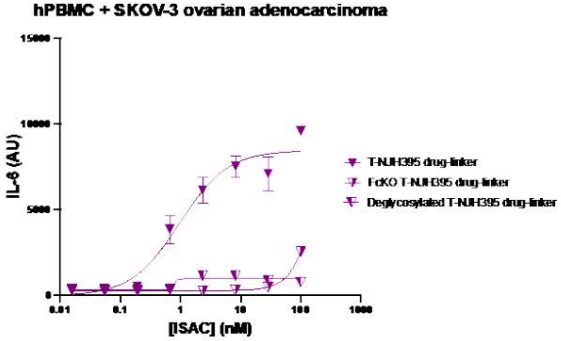


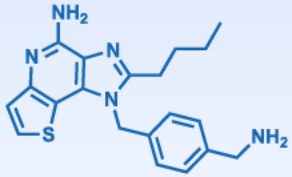

### 4 Fc-knockout inhibits activity




hPBMCs = human blood peripheral mononuclear cells

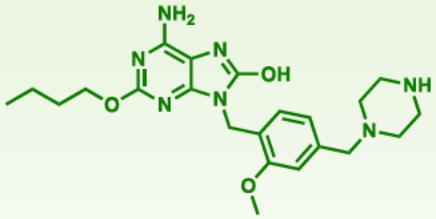
# Zymeworks has Evaluated Two Scaffold Classes as ISAC Payloads

## 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

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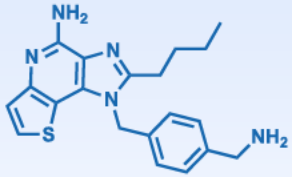

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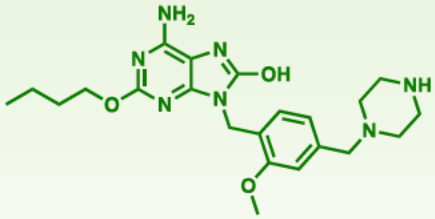

- Pure TLR7 agonist
- Broadly explored as small molecule agonist

- 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

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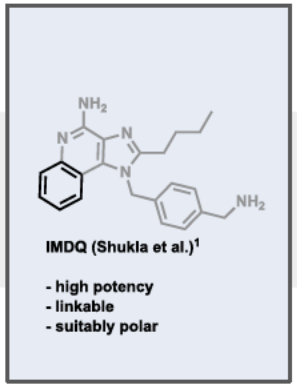
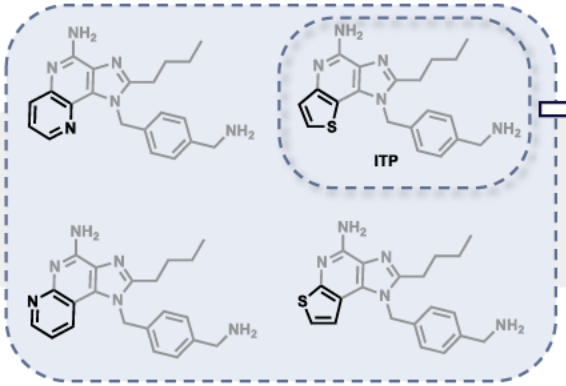
*Starting point*

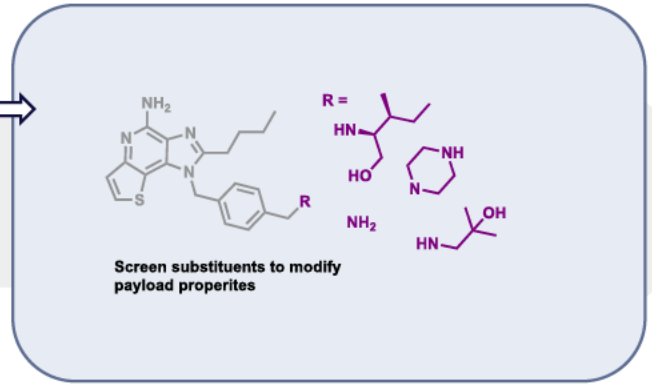



Scaffold diversification

*Scaffold evaluation*




*Substituent evaluation*




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

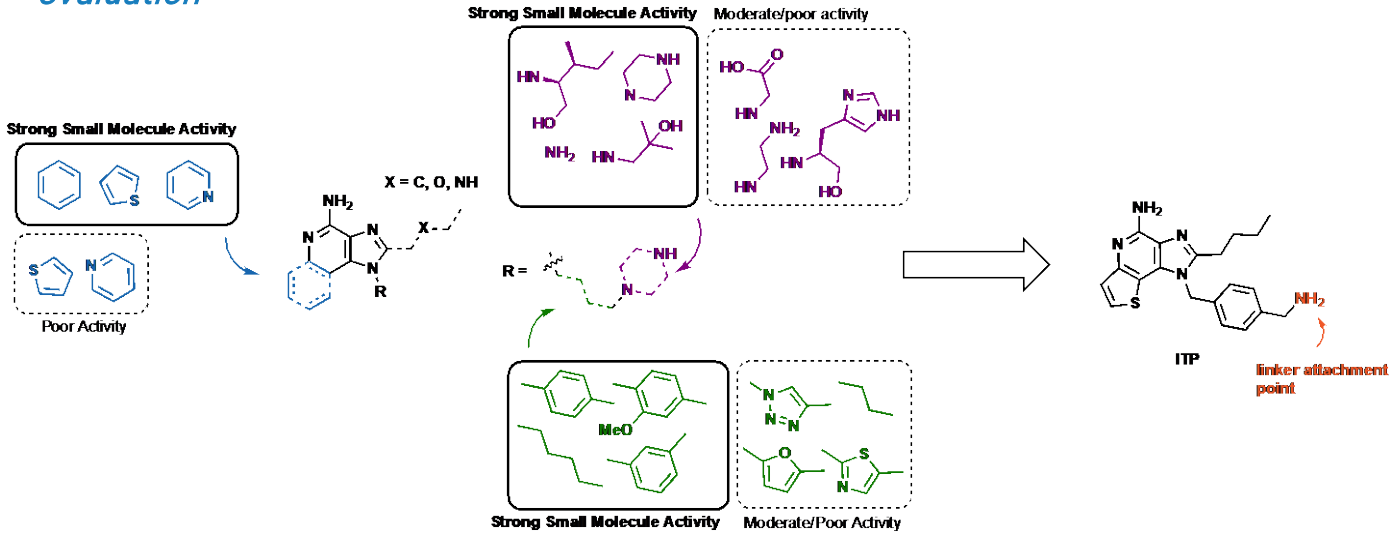
*SAR learnings:*

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

*Benzo-variant evaluation*

*Substituent optimization*

*Small molecule lead*




*manuscript submitted*

# Novel Imidazoquinoline-type Drug-linkers Generate Trastuzumab-isacs with Favorable Biophysical Characteristics

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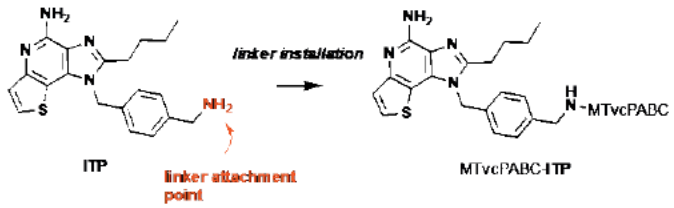
## *Drug-linker generation*

***Drug-linkers were synthesized from novel payloads***

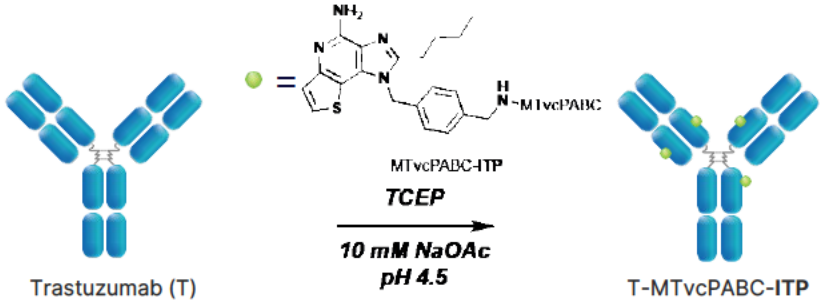
- Cleavable linker system

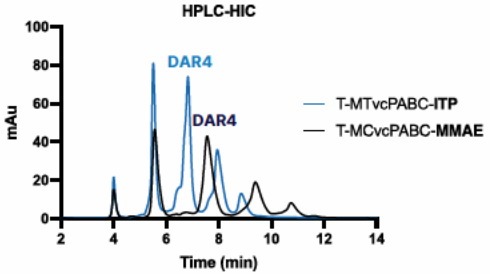



## *ADC generation and characterization*

***ISACs were generated***

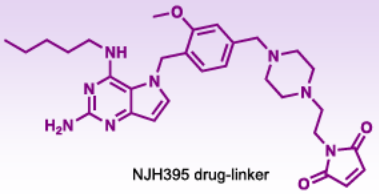
- Trastuzumab
- Cysteine conjugation
- Stochastic DAR = 4



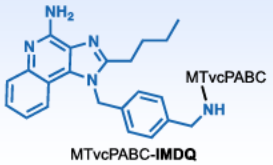



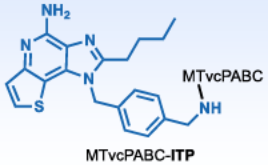
# In a Murine In Vitro Assay, Comparable Activity was Observed for Imidazoquinoline and Pyrrolopyrimidine Isacs

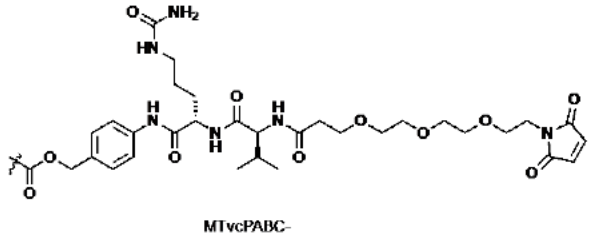
## Pyrrolopyrimidine (benchmark)



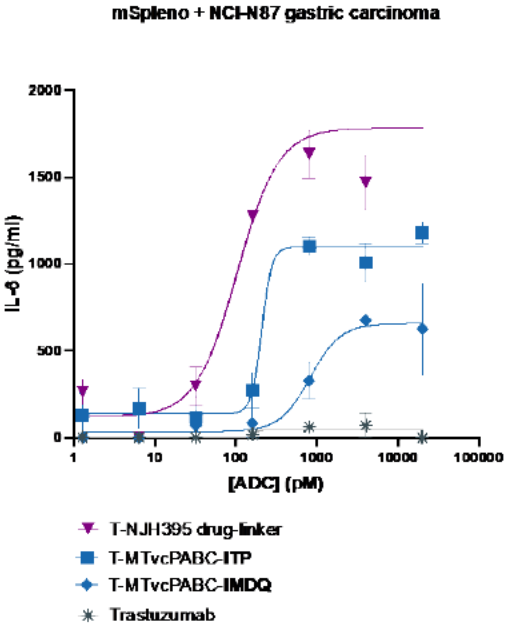

## Imidazoquinoline



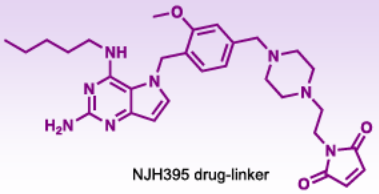

mSpleno = murine splenocytes



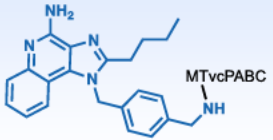

| | Murine activity | Human activity |
|---|---|---|
| Pyrrolopyrimidine | ✅ | |
| Imidazoquinoline | ✅ | |

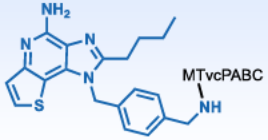
# In a Murine CDX Model, Comparable Activity was Observed from Imidazoquinoline and Pyrrolopyrimidine Isacs

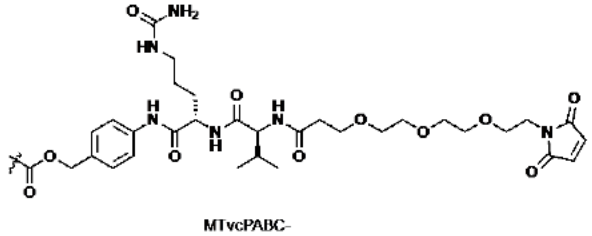
## Pyrrolopyrimidine (benchmark)



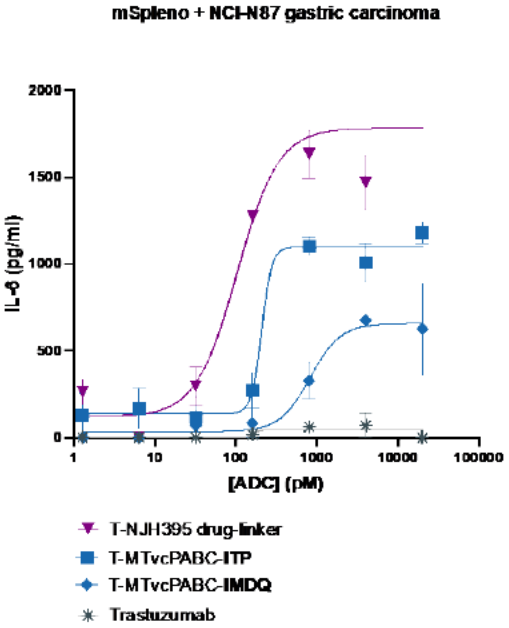

## Imidazoquinoline

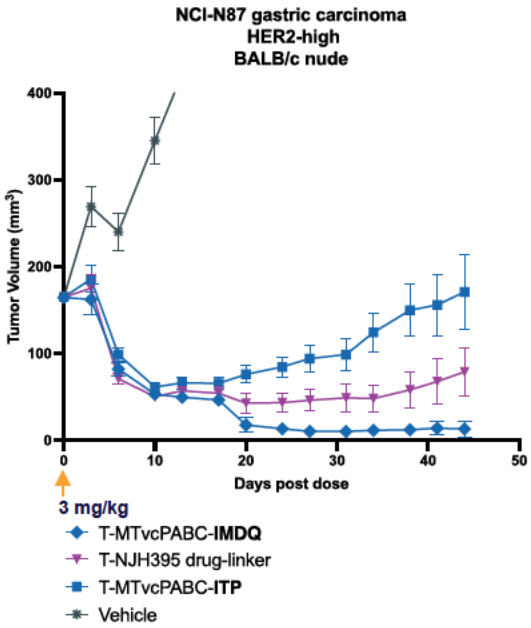



mSpleno = murine splenocytes

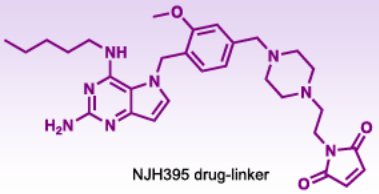


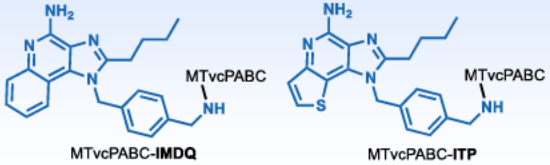

| | Murine activity | Human activity |
|---|---|---|
| Pyrrolopyrimidine | ✅ | |
| Imidazoquinoline | ✅ | |

# Purine Isacs Showed Consistent and Robust Responses Across In Vitro Assays with Human and Murine Primary Cells

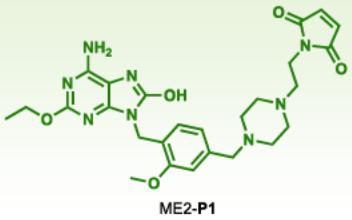
## Pyrrolopyrimidine (benchmark)

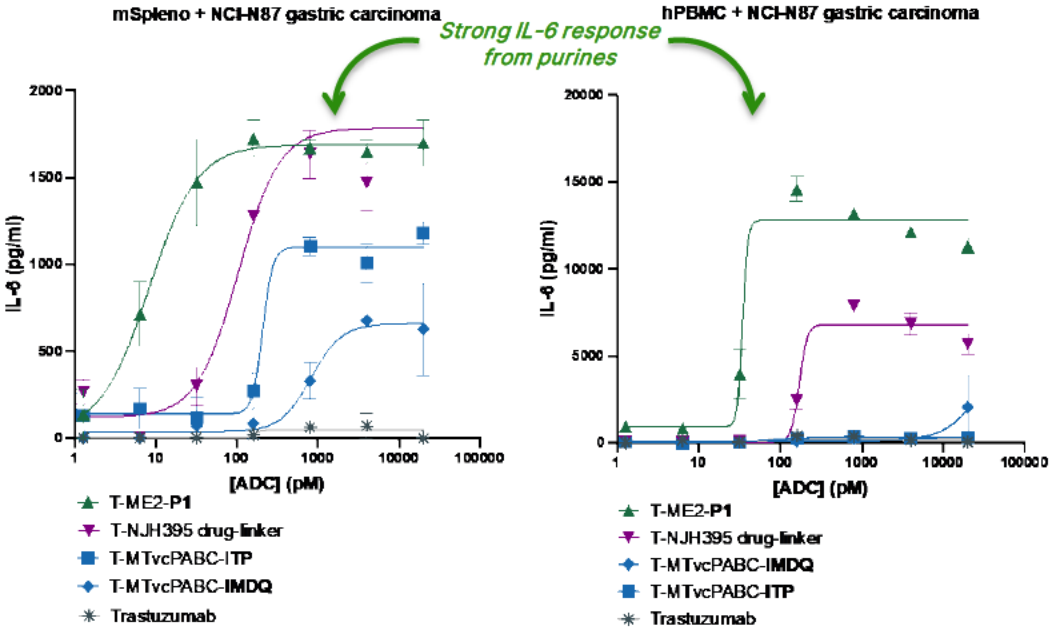



## Imidazoquinoline




## Purine



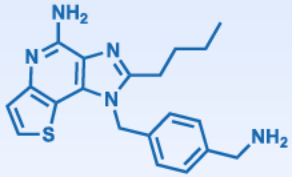




| | Murine activity | Human activity |
|---|---|---|
| Pyrrolopyrimidine | ✅ | ✅ |
| Imidazoquinoline | ✅ | ? |
| Purine | ✅ | ✅ |

mSpleno = murine splenocytes
hPBMCs = human blood peripheral mononuclear cells

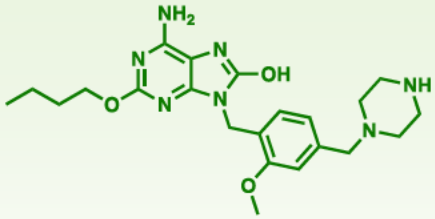
# Zymeworks has Evaluated Two Scaffold Classes as ISAC Payloads

## Imidazothienopyridine




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

## Purine

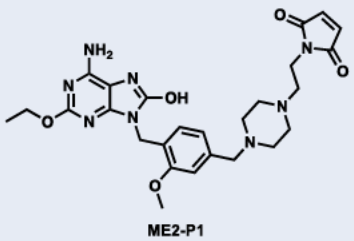



- Pure TLR7 agonist
- Broadly explored as small molecule agonist

- 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

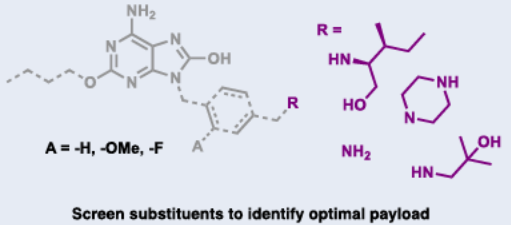
*Scaffold validation*




ME2-P1

1. Identify scaffolds with strong species cross-reactivity

*Substituent evaluation*

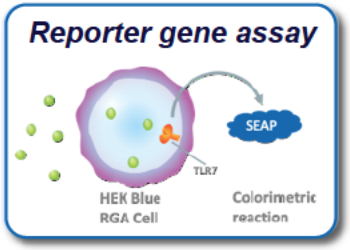


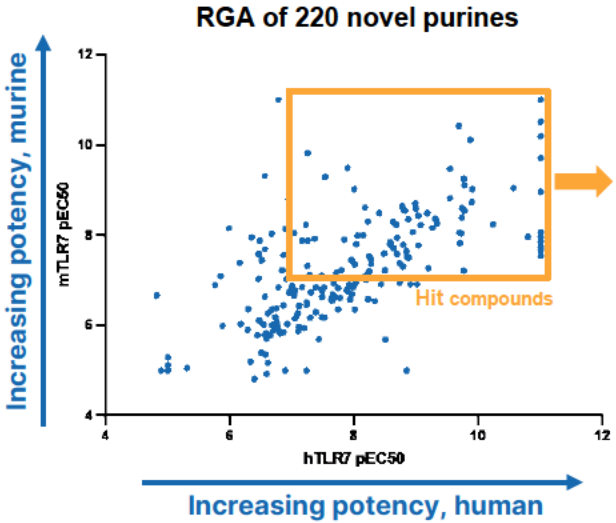

Screen substituents to identify optimal payload

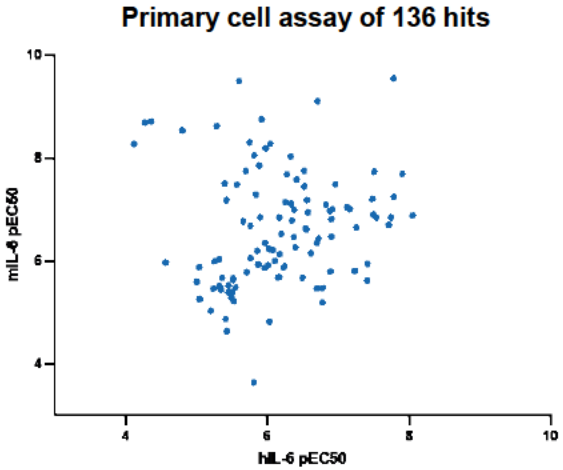
2. Optimize the substituent portion for use as a bioconjugate

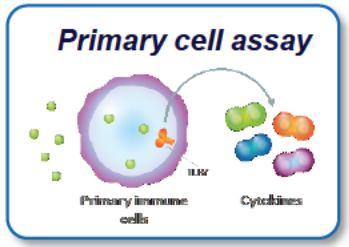
*manuscript in preparation*

# Small Molecules were Screened and Selected for ISAC Conjugation Based on Potency and Structural Diversity



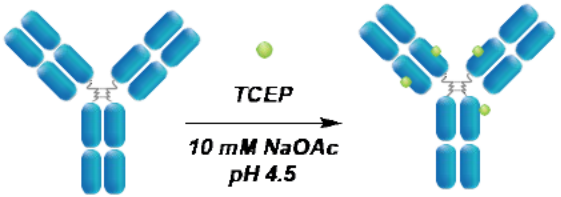




- 220 novel small molecule purines were synthesized
- Hit compounds (pEC50 > 7 in both assays) were evaluated against human and murine primary immune cells
- Drug-linkers were generated from the 40 most promising compounds

# Novel Purine Drug-linkers Generate Trastuzumab-isacs with Favorable Biophysical Characteristics

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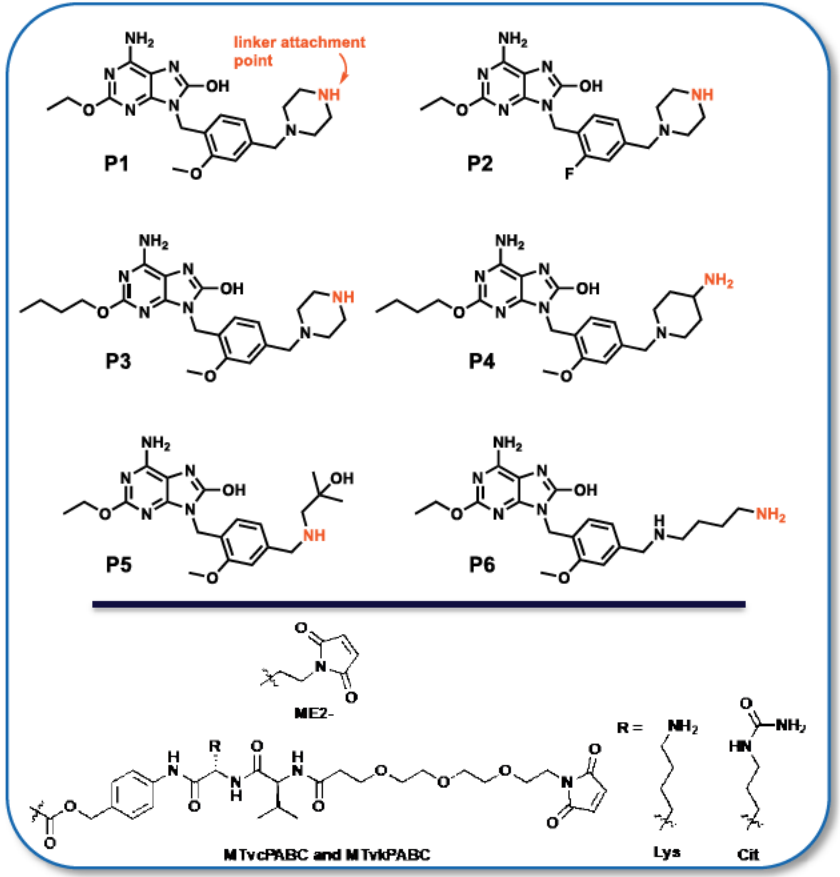
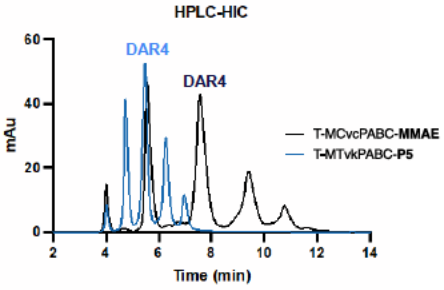
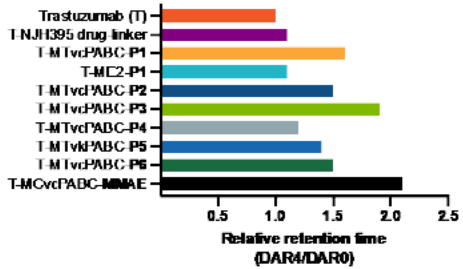


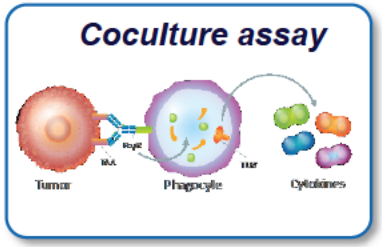

- Purine drug-linkers were conjugated to trastuzumab to achieve an average drug-to-antibody ratio (DAR) of 4

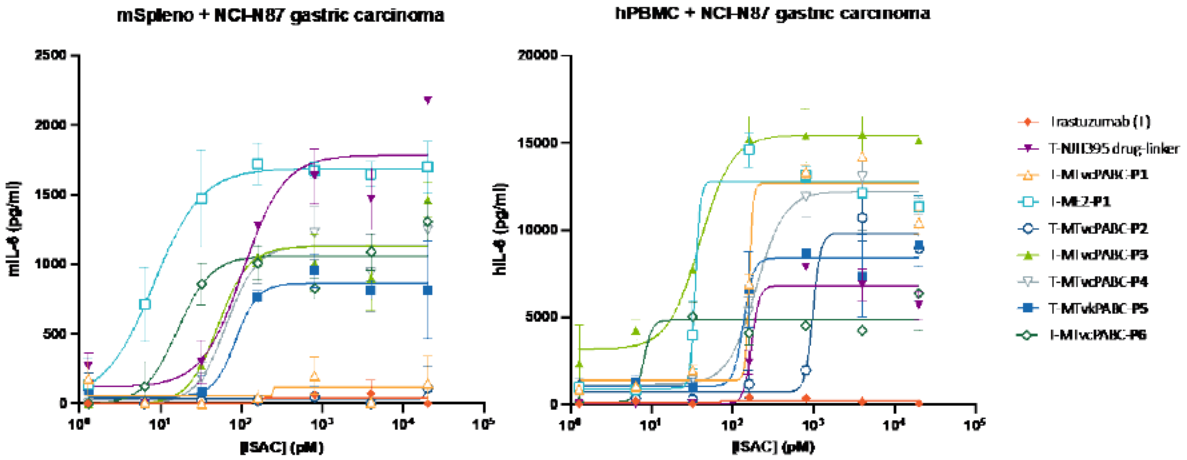



- The resulting ISACs demonstrated low aggregation and acceptable hydrophobicity

# Purine Isacs Drive Potent Immune Response in both Mouse and Human Coculture Systems



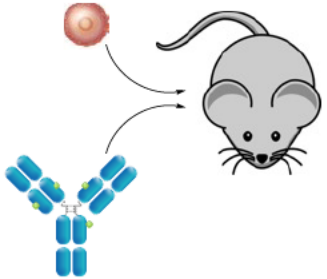

- Human PBMCs or murine splenocytes were cocultured with N87 tumor cells in the presence of indicated ISACs.
- ISACs capable of inducing high levels of IL-6 from cocultures of tumor cells and primary immune cells were selected for in vivo studies

mSpleno = murine splenocytes
hPBMCs = human blood peripheral mononuclear cells

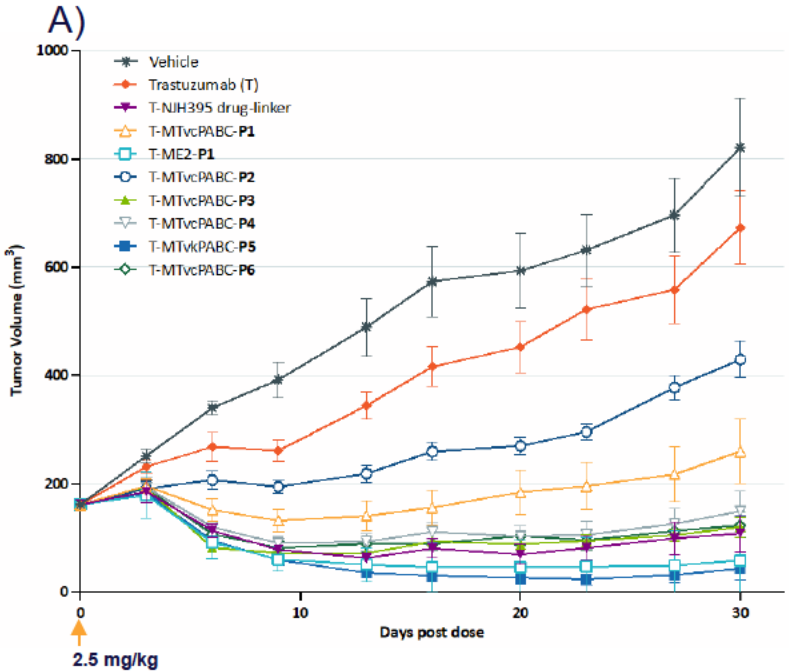
# Novel Purine Isacs Show Similar In Vivo Efficacy to T-NJH395 Benchmark

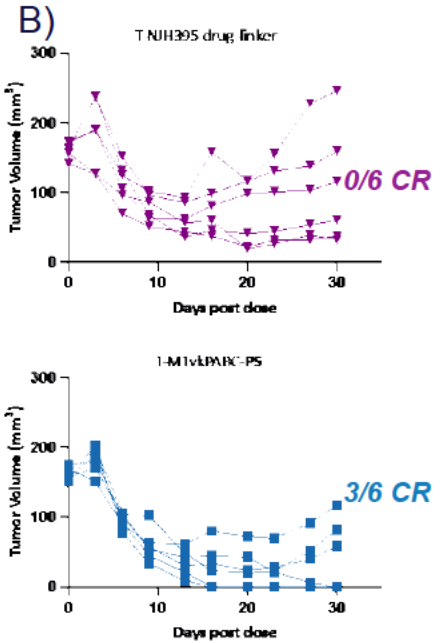
*In vivo cell-derived xenograft (CDX)*

*Engraft tumor*



*Administer ISAC at 2.5 mg/kg*



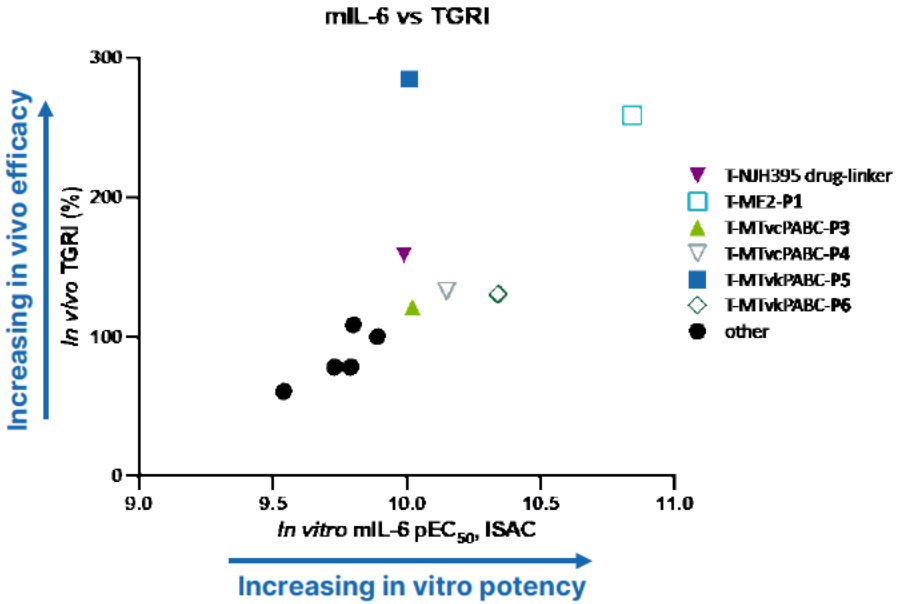

Antitumor activity of purine-based TLR7 agonists conjugated to trastuzumab (DAR = 4) in an NCI-N87 tumor cell-line derived xenograft BALB/c nude model.

# 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:

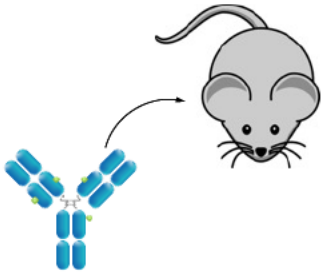
$$TGRI = [1 - \frac{tumor\ growth\ rate\ kinetic\ of\ treated\ group}{tumor\ growth\ rate\ kinetic\ of\ control\ group}] \ x\ 100$$

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

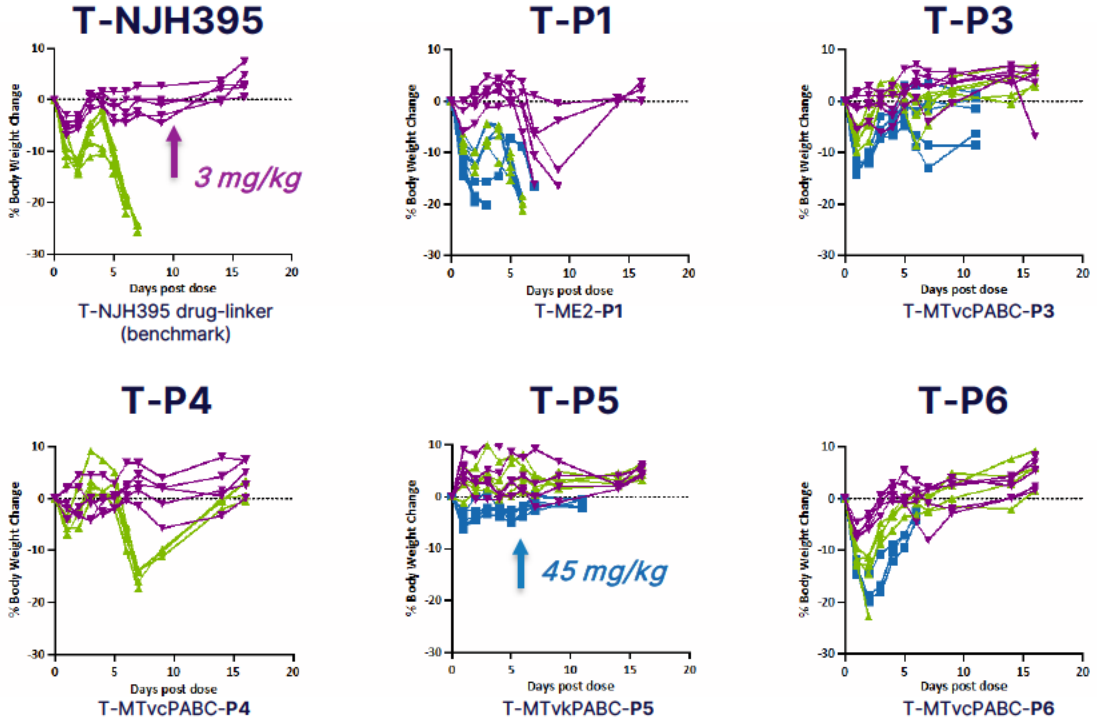


# Novel Purine Isacs Show Superior In Vivo Tolerability to T-NJH395 Benchmark

*Murine tolerability (BALB/c mice)*



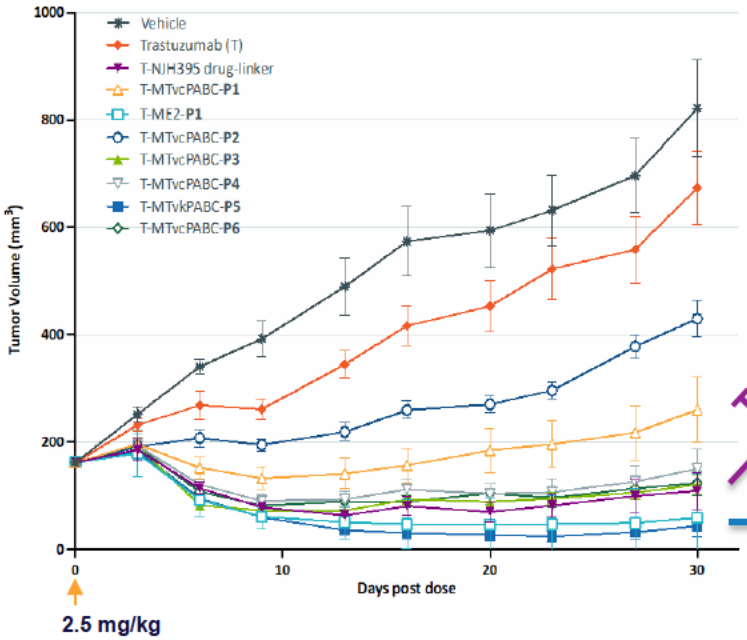
*Administer ISAC up to 45 mg/kg*




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

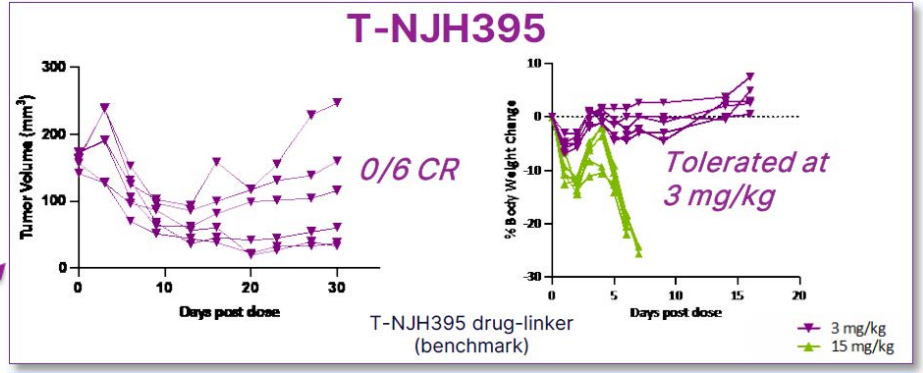


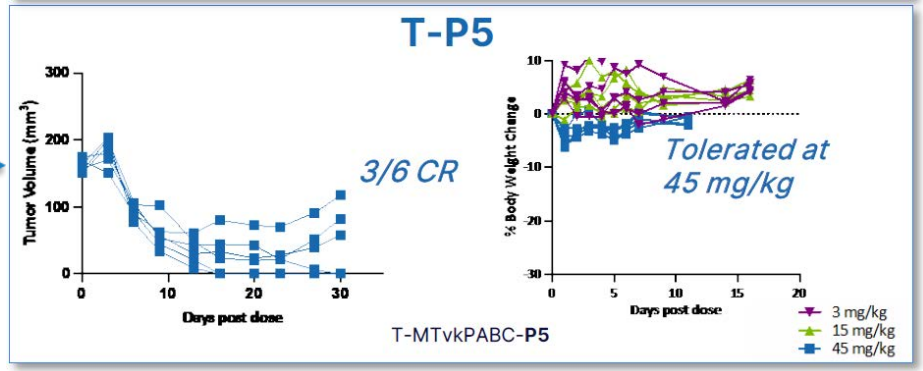

T-NJH395

T-P5

Efficacy

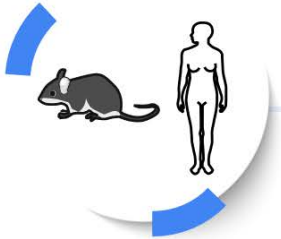
Tolerability




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

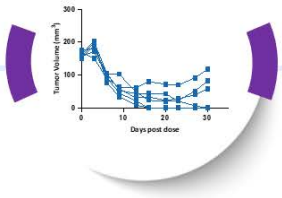
# Purine-based Isacs have Demonstrated Compelling Preclinical Activity



## Cross-species Activity

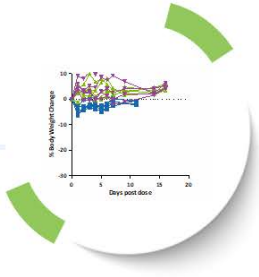
Purine-based ISACs showed **strong activity on both murine and human immune cells** in vitro.

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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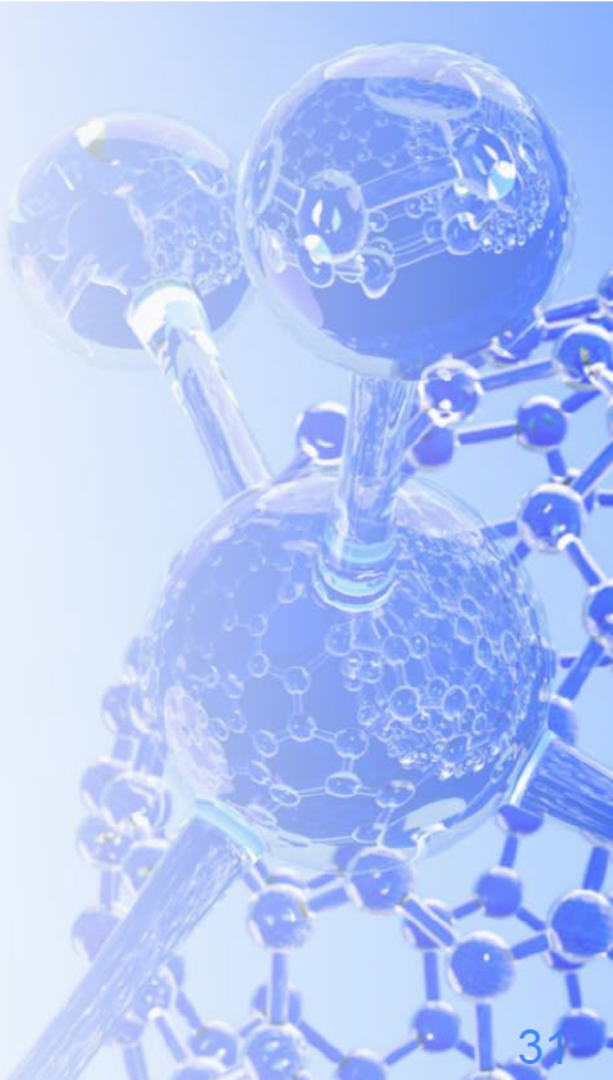
- Gerry Rowse
- Joel Smith
- Florian Heinkel
- Tong Ding
- Cathy Dang

# Thank You



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