Development of TLR-ADCs Using Novel Selective TLR7, TLR8 or TLR7/8 Dual Agonists as Payloads

HN Yu, SS Wang, W Geng, K Qin, SL Wang, G Massimini, R Lou

CanWell Pharma Inc., Woburn, MA, USA. karl@canwellpharma.com; info@canwellpharma.com

Background

The activation of Toll-like receptors (TLRs) is one of the key components participating in the 1st line of host immune defense against foreign pathogens such as viruses and bacteria. However, in the cancer microenvironment, several types of immune cells involve in this mechanism, particularly, macrophages and dendritic cells (DCs), play crucial roles via activation of TLRs in mediating innate immune responses and promoting adaptive immunity against tumors. While treatments of various TLR agonists by either local (intra-tumoral, IT) or systemic (IV or ADC) administration has shown some encouraging antitumoral activities in early clinical trials, challenges remain on development of the next generation TLR based therapies with robust immune stimulating potencies and satisfactory safety profiles. Based on our proprietary large library of TLR molecules, CanWell has developed comprehensive TLR programs to address these challenges: (1) CAN1012, an IT-delivered high TLR7-selective agonist (phase I clinical trials ongoing in the US and China, NCT04987112, NCT05580991), and (2) TLR-ADC therapies, including a number of pre-clinical TLR-ADCs with different TLR7 or TLR8 selectivity.
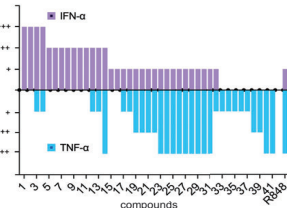
Novel selective TLR7, TLR8 or dual TLR7/8 agonists were designed, synthesized and screened. Compounds were selected based on TLR selectivity and in-vitro hPBMC functional assays. They were coupled to a collection of cleavable and non-cleavable linkers. The resulting linker-payloads (LPs) were conjugated with antibodies (anti-Her2 mAb, anti-PD-L1 mAb, etc). The results are presented with molecule selectivity, cytokine activation profile, conjugation chemistry, in vitro stability, in-vivo PK and efficacy in mouse tumor models. These findings provide robust support for developing the potential next generation TLR-ADCs against cancers by applying our TLR7/8 linker-payloads. Further developments is currently ongoing using these molecules.

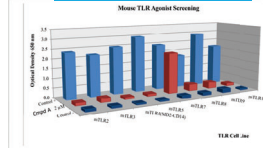## A diverse selective TLR7/8 compounds has been synthesized
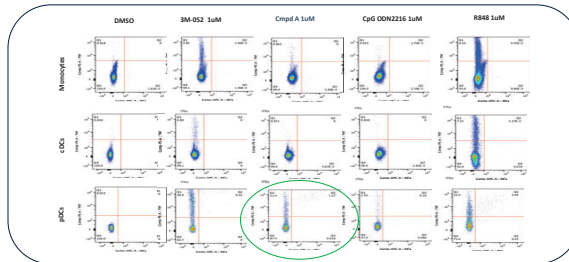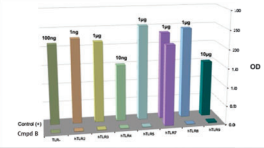
Human PBMC assay
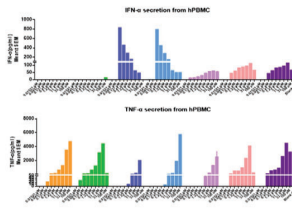
Exemplary compound collection

Novel selective TLR7, TLR8 or dual TLR7/8 agonists were designed, synthesized and screened in in vitro hPBMC functional assays.

Highly differentiated compounds: TLR7 selectivity, selective pDC activation
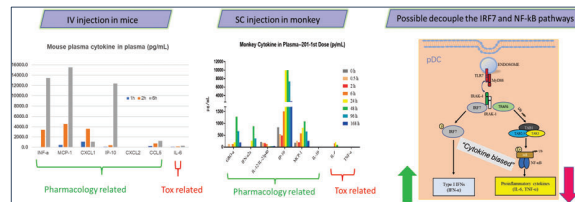
Mouse TLR agonist screening

Human TLR agonist screening

Some compounds were profiled against mouse and human TLR panels, showing different TLR7/8 selectivity profile.
Compound A was demonstrated to selectively activate plasmacytoid dendritic cells (pDC).

"Cytokine-biased" compound – safer TLR7 agonist

## TLR7/8 linker-payloads were conjugated to different monoclonal antibodies

Exemplary ADCs prepared from TLR7/8 agonist linker payloads
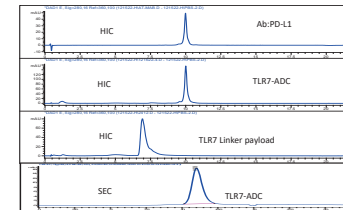
ADC Example	mAb	Linker Payload	Agg%	UmAb%	DAR
22	Trastuzumab	10	4.6	6.6	4.3
23	Trastuzumab	11	2.4	6.5	4.6
24	Trastuzumab	12	6.8	3.1	5.0
25	Trastuzumab	13	8.5	3.8	4.8
26	Avelumab	10	<1.0	3.8	4.5
27	Avelumab	11	<1.0	4.4	4.5
28	Avelumab	12	2.9	2.2	4.9
29	Avelumab	13	3.4	1.2	5.2
30	Trastuzumab	15	1.4	4.9	4.6
31	Avelumab	15	1.3	5.1	3.9
32	Trastuzumab	14	2.1	3.3	4.5
33	Avelumab	14	<1.0	5.3	4.6
34	mouse anti-human HER2 Ab	18	3.0	8.8	4.9
35	mouse anti-human HER2 Ab	19	1.9	2.1	5.6
36	mouse anti-human HER2 Ab	20	1.6	4.2	4.3
37	mouse anti-human HER2 Ab	21	1.6	6.5	4.3
38	Anti-mouse PD-L1 (B7-H1)	18	6.3	7.7	5.4
39	Anti-mouse PD-L1 (B7-H1)	19	4.4	10.8	4.8
40	Anti-mouse PD-L1 (B7-H1)	20	0.7	5.5	5.0
41	Anti-mouse PD-L1 (B7-H1)	21	1.4	9.8	4.9
42	Avelumab	20	1.5	8.6	3.6
43	Avelumab	21	1.0	9.1	3.5
44	Atezolizumab	21	0.0	0.0	5.7

TLR linker payloads are hydrophilic. The conjugation chemistry was facile and scalable.

TLR 7/8 ADCs – Favorable PK and efficacy in vivo

ADC	DAR	Mouse plasma stability
Avelumab -TLR7	3.5	100% remain @ 168 h
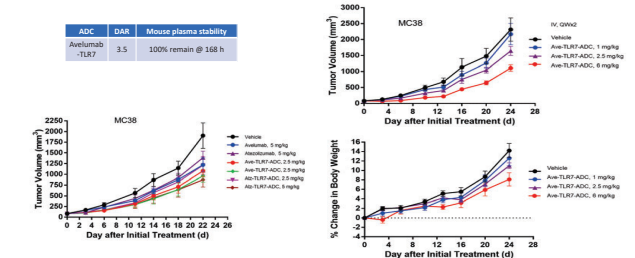
Conclusions

- ☐ Novel selective TLR7, TLR8 or dual TLR7/8 agonists library were designed, synthesized
- ☐ Some compounds are highly differentiated: TLR7 or 8 selective; selective activate pDC; and "cytokine-biased", which might result in safer TLR molecules
- ☐ TLR linker-payloads are hydrophilic
- ☐ Conjugate chemistry has been validated with cleavable or non-cleavable linkers
- ☐ TLR-ADC has favorable DMPK properties
- ☐ In vivo efficacy has been demonstrated
- ☐ Partnering is welcome!

CANWELL