Scientific Poster Session

n-lorem FOUNDATION

Nano-rare Patient Colloquium 2025

Monday, October 20 | 5:15 – 6:15 pm EST

Phosphorothioate Antisense Oligonucleotide Induced Innate Immune Activation is Terminated by Tryptophan Oxidation Products

Phosphorothioate antisense oligonucleotides (PS-ASOs) can activate the innate immune response via toll-like receptor 9 (TLR9), creating safety challenges in the clinic. Our research has uncovered how the PS-ASO-induced innate immune response is terminated. This work significantly advances our understanding of PS-ASOs, enabling the development of drugs that are better tolerated to improve outcomes for patients.

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Senior Scientist, n-Lorem

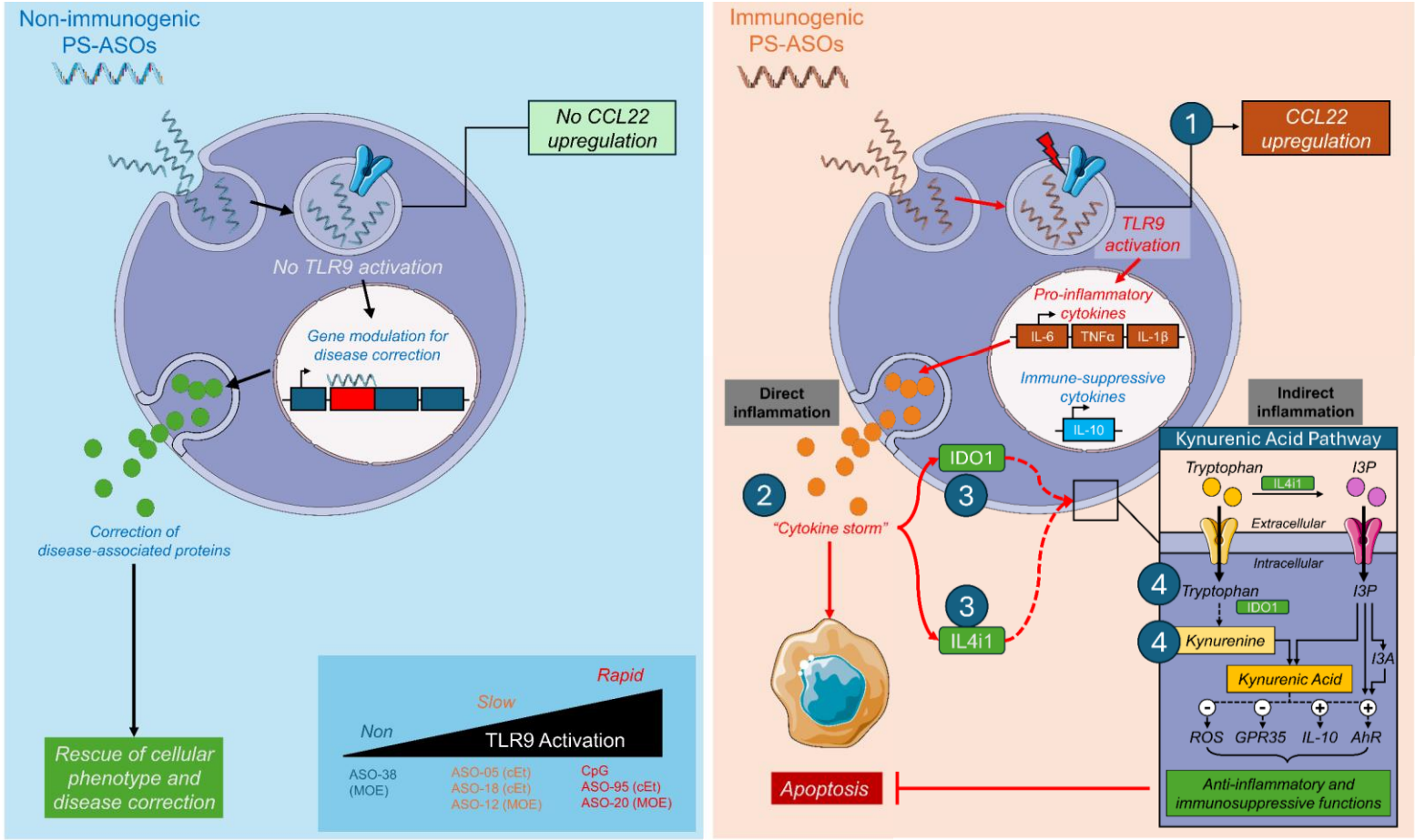
Hosted by:

Biogen

Introduction

- The molecular mechanisms underpinning PS ASOs innate immune activation are well known and have benefitted nano-rare patients enormously
- Termination of the innate immune response after PS-ASO activation remains elusive
- Tryptophan oxidation, via the kynurenine pathway and indole oxidative pathway are key mechanisms of innate immune termination
- Bioactive metabolites of these pathways may quench the immune response, and represent a potential anti-immunogenic co-therapy
- We sought to explore the role of these metabolites in response to both immunogenic and non-immunogenic PS-ASOs
- And subsequently we uncovered that
  - Kinetics and signal intensity of PS ASO induced innate immune responses vary between cell systems and between PS ASOs
  - Trp oxidation enzymes, including IDO1 and IL4I1, are upregulated by PS ASO-mediated innate immune activation
  - Kinetics and scale of the induction of IDO1 and IL4I1 vary, and
  - Silencing IDO1 increases PS ASO innate immune activation

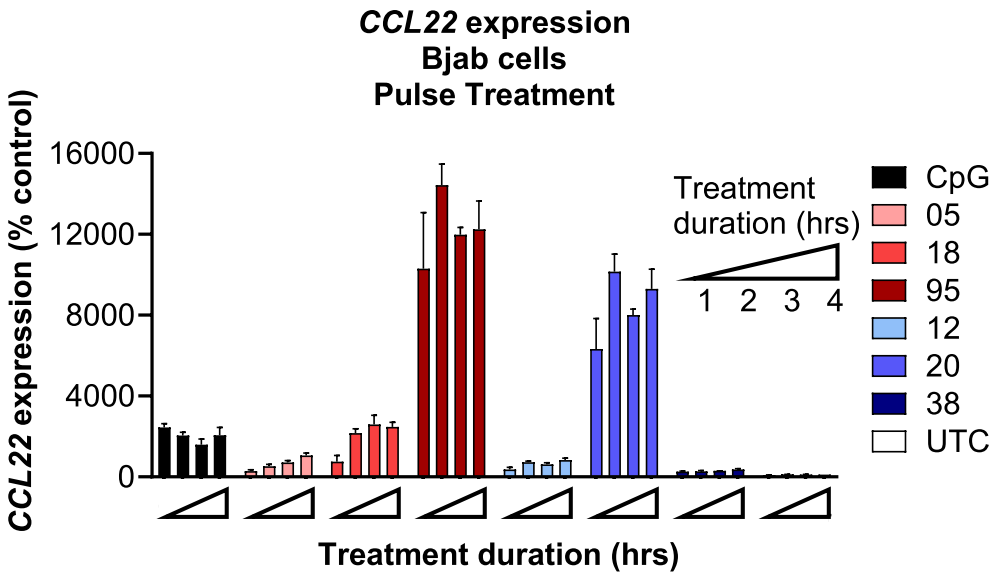
Abstract

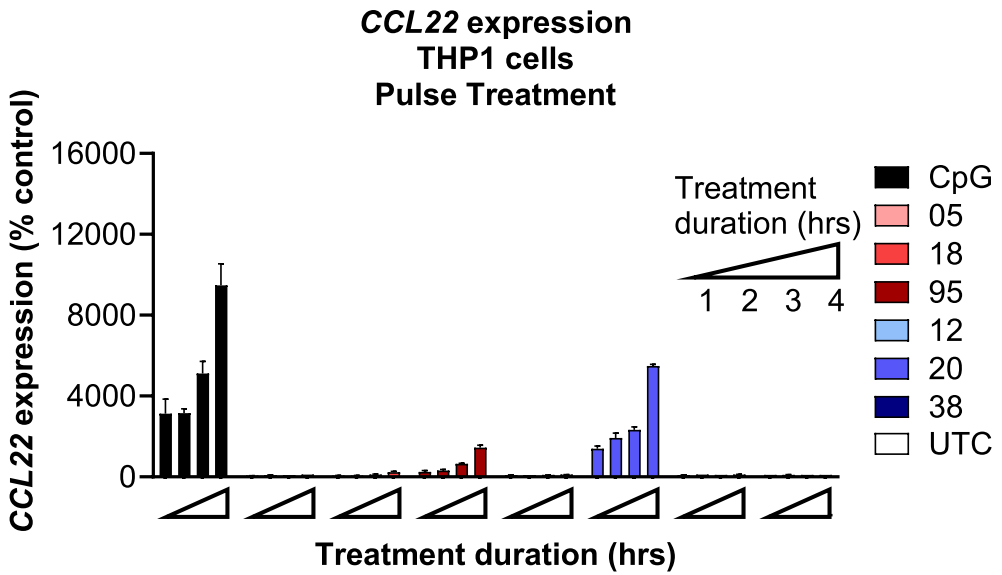

Non-immunogenic
PS-ASOs
No CCL22 upregulation
No TLR9 activation
Gene modulation for disease correction
Correction of disease-associated proteins
Rescue of cellular phenotype and disease correction
Non
Slow
Rapid
TLR9 Activation
ASO-38 (MOE)
ASO-05 (cEt)
ASO-18 (cEt)
ASO-12 (MOE)
CpG
ASO-95 (cEt)
ASO-20 (MOE)
Immunogenic
PS-ASOs
1
CCL22 upregulation
TLR9 activation
Pro-inflammatory cytokines
IL-6
TNFα
IL-1β
Immune-suppressive cytokines
IL-10
Direct inflammation
Indirect inflammation
Kynurenic Acid Pathway
2
"Cytokine storm"
IDO1
3
IL4i1
Tryptophan
I3P
IL4i1
Extracellular
Intracellular
4
Tryptophan
IDO1
I3P
4
Kynurenine
I3A
Kynurenic Acid
ROS
GPR35
IL-10
AhR
Apoptosis
Anti-inflammatory and immunosuppressive functions

Results

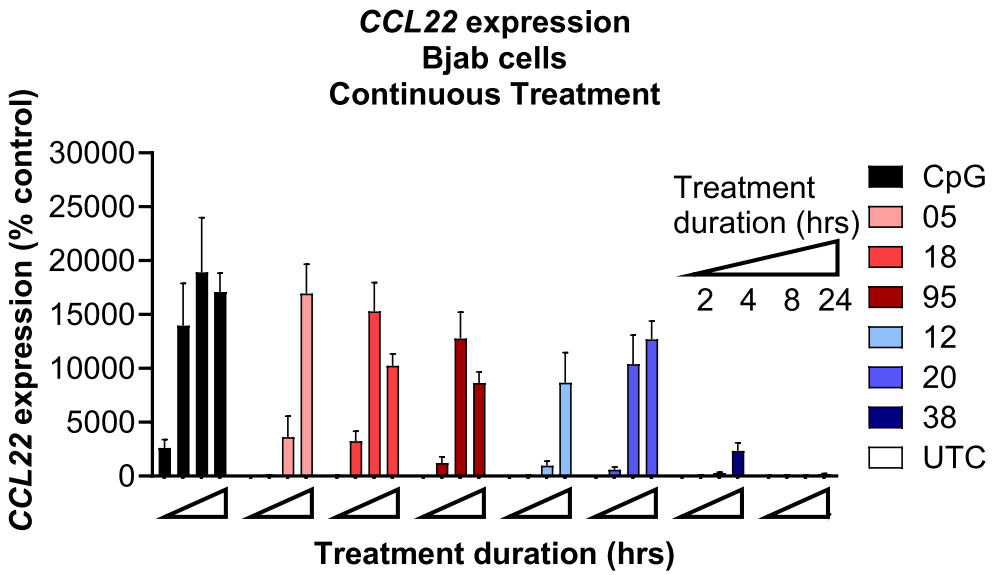
PS ASO immunogenicity varies between different cell systems

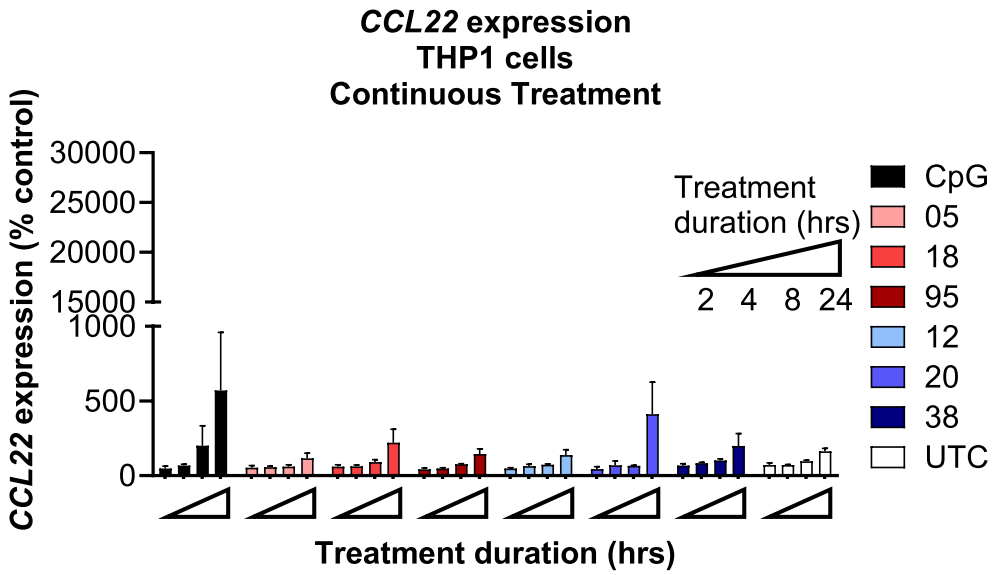
A


CCL22 expression
Bjab cells
Pulse Treatment
CCL22 expression (% control)
16000
12000
8000
4000
0
Treatment duration (hrs)
1 2 3 4
CpG
05
18
95
12
20
38
UTC
Treatment duration (hrs)

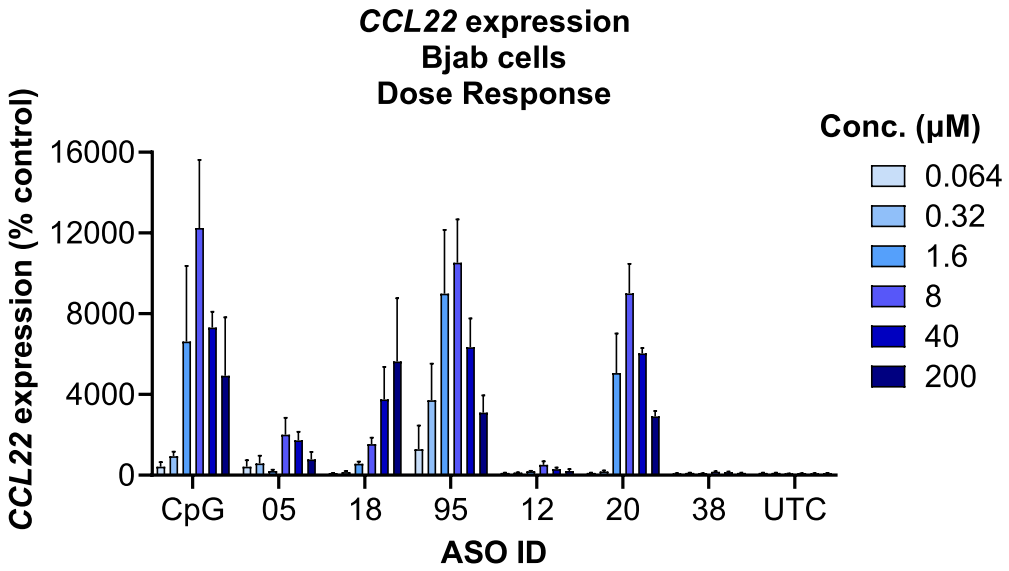

CCL22 expression
THP1 cells
Pulse Treatment
CCL22 expression (% control)
16000
12000
8000
4000
0
Treatment duration (hrs)
1 2 3 4
CpG
05
18
95
12
20
38
UTC
Treatment duration (hrs)

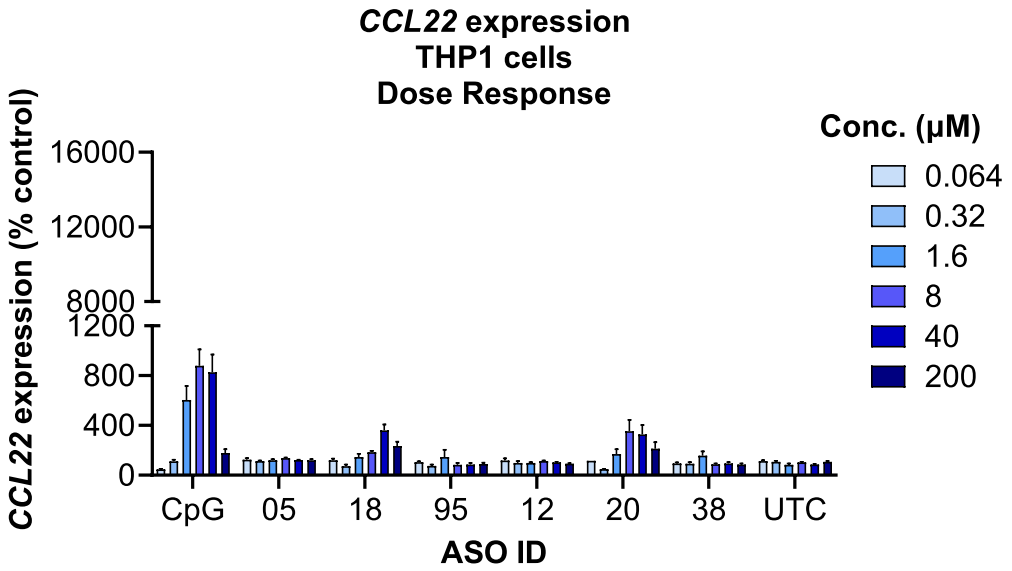
B


CCL22 expression
Bjab cells
Continuous Treatment
CCL22 expression (% control)
30000
25000
20000
15000
10000
5000
0
Treatment duration (hrs)
2 4 8 24
CpG
05
18
95
12
20
38
UTC
Treatment duration (hrs)


CCL22 expression
THP1 cells
Continuous Treatment
CCL22 expression (% control)
30000
25000
20000
15000
1000
500
0
Treatment duration (hrs)
2 4 8 24
CpG
05
18
95
12
20
38
UTC
Treatment duration (hrs)

C

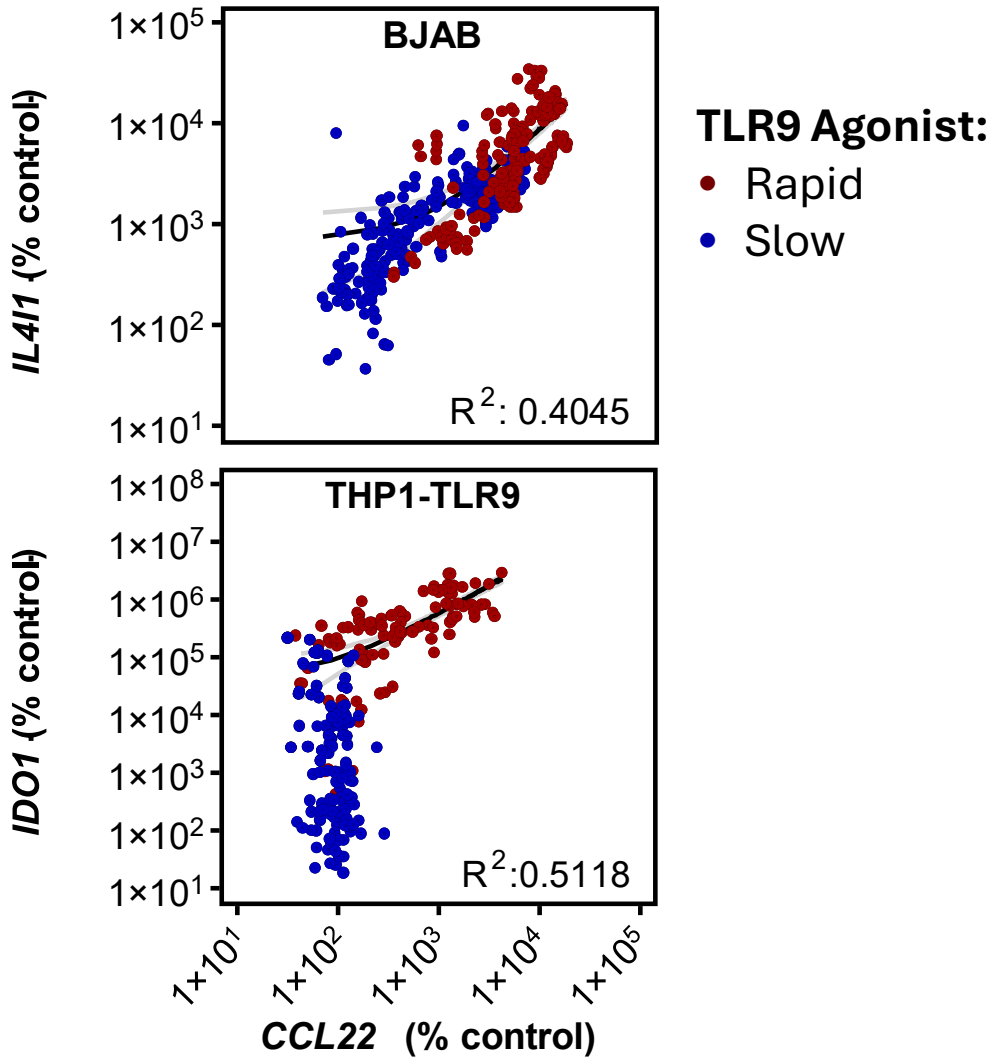

CCL22 expression
Bjab cells
Dose Response
CCL22 expression (% control)
16000
12000
8000
4000
0
CpG
05
18
95
12
20
38
UTC
ASO ID
Conc. (μM)
0.064
0.32
1.6
8
40
200


CCL22 expression
THP1 cells
Dose Response
CCL22 expression (% control)
16000
12000
8000
1200
800
400
0
CpG
05
18
95
12
20
38
UTC
ASO ID
Conc. (μM)
0.064
0.32
1.6
8
40
200

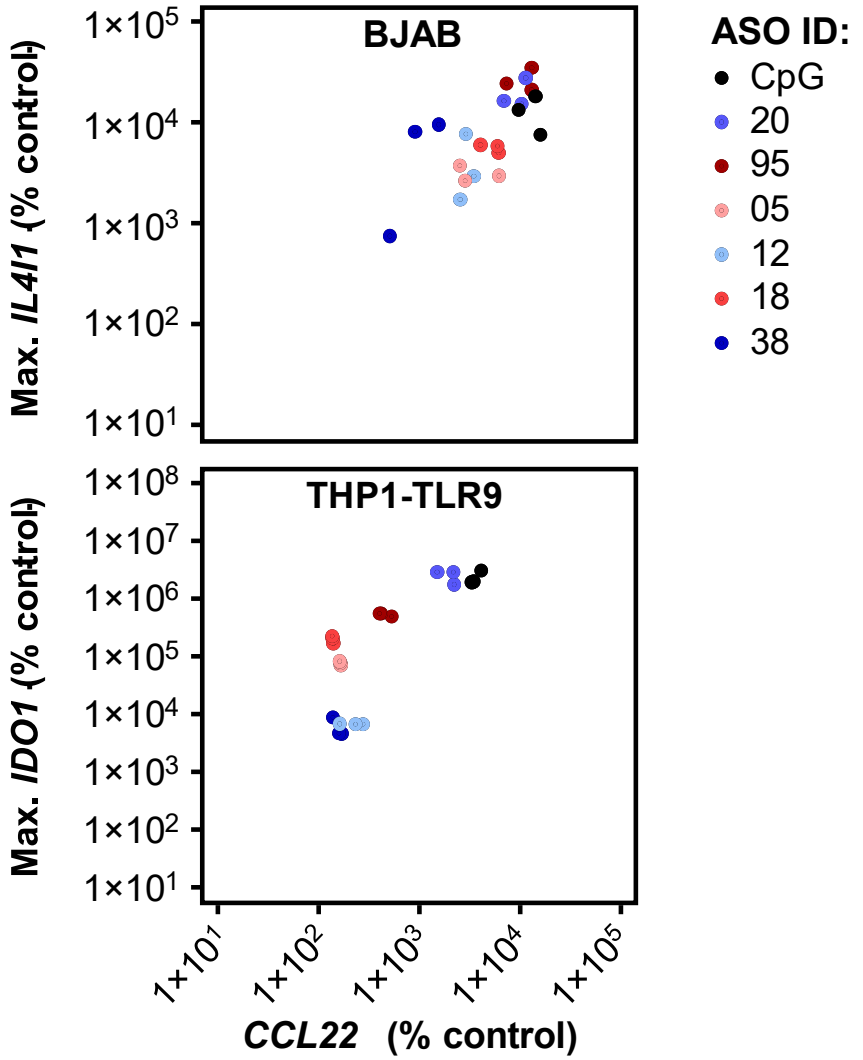
Results

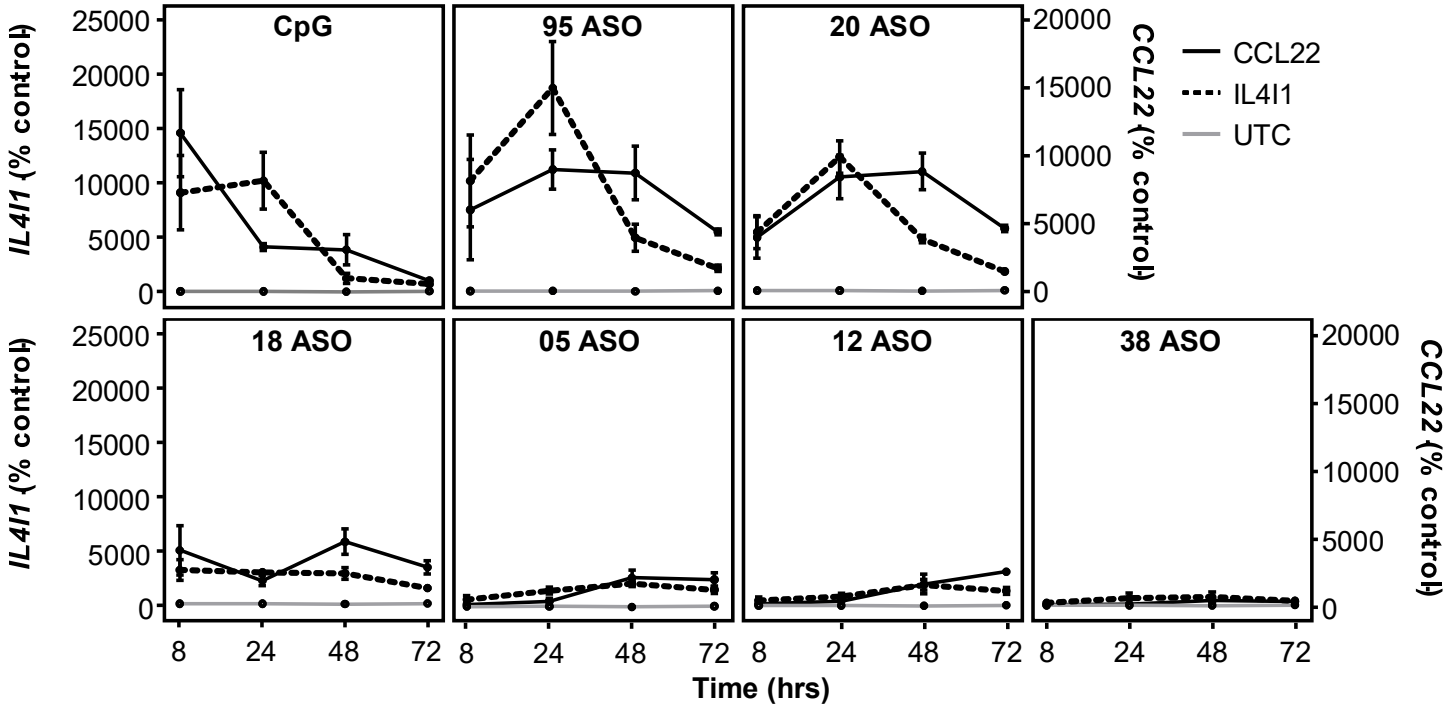
PS ASO TLR9 agonists upregulate Trp metabolism enzymes IDO1 and IL41

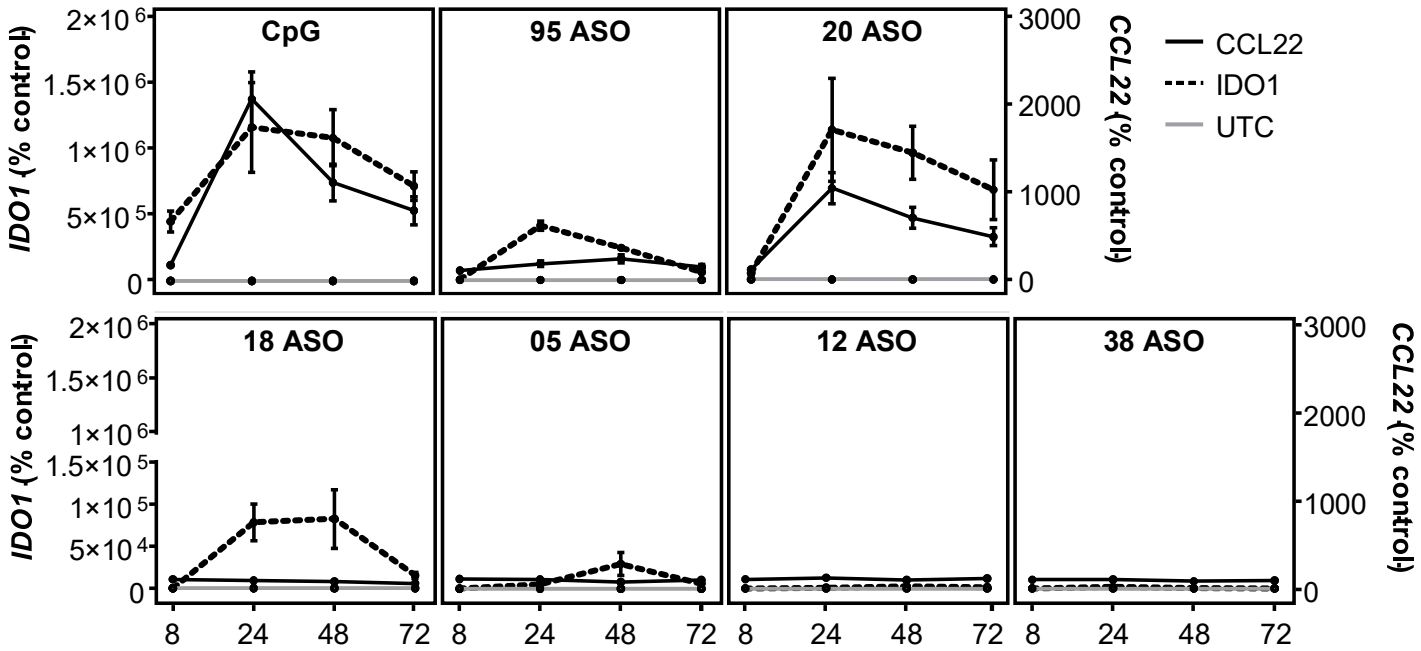
A


BJAB
THP1-TLR9
IL4I1 (% control)
IDO1 (% control)
CCL22 (% control)
R2: 0.4045
R2:0.5118
TLR9 Agonist:
Rapid
Slow

B

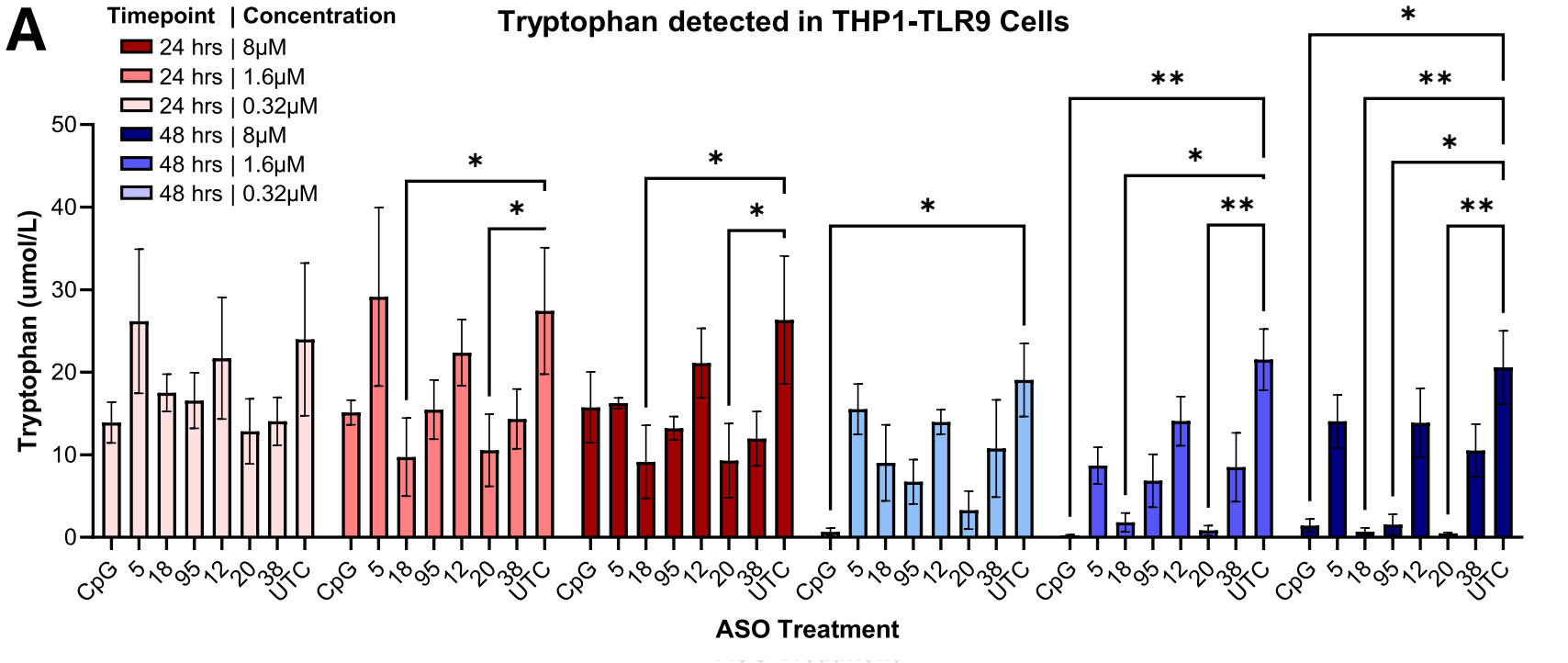

BJAB
THP1-TLR9
Max. IL4I1 (% control)
Max. IDO1 (% control)
CCL22 (% control)
ASO ID:
CpG
20
95
05
12
18
38

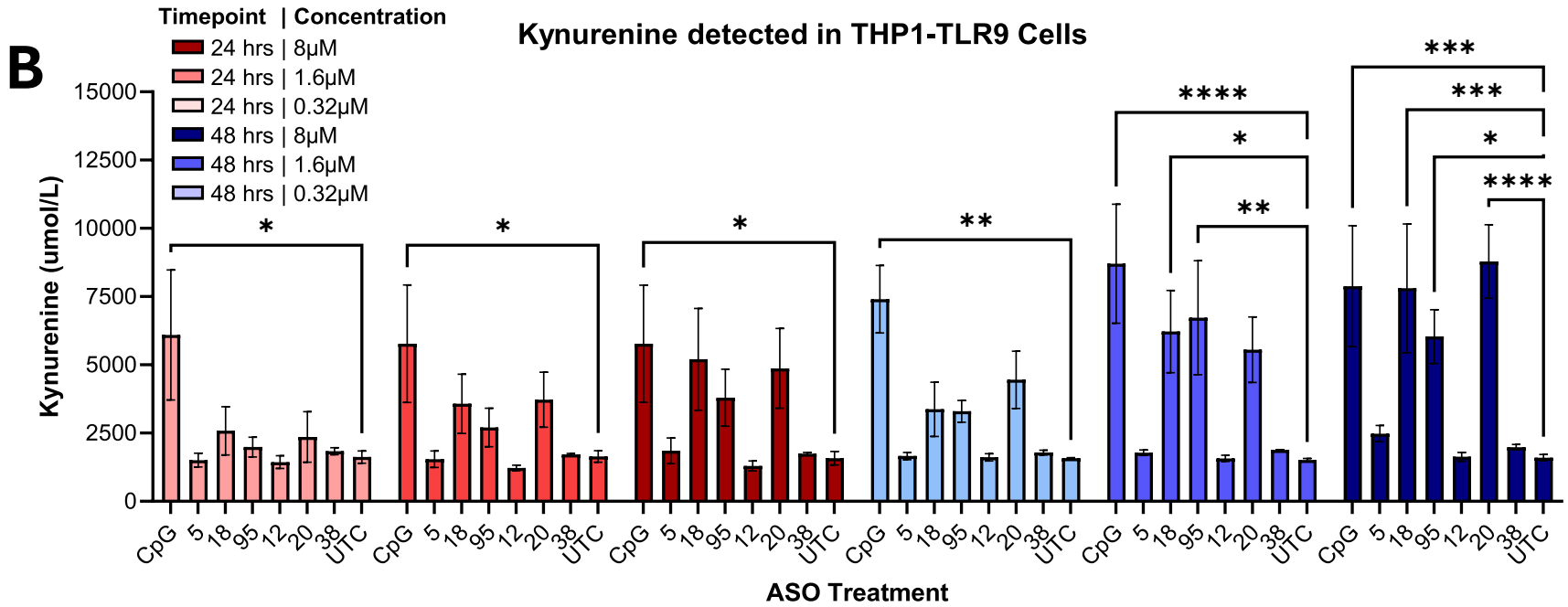

CpG
95 ASO
20 ASO
18 ASO
05 ASO
12 ASO
38 ASO
IL4I1 (% control)
CCL22 (% control)
CCL22
IL4I1
UTC
Time (hrs)

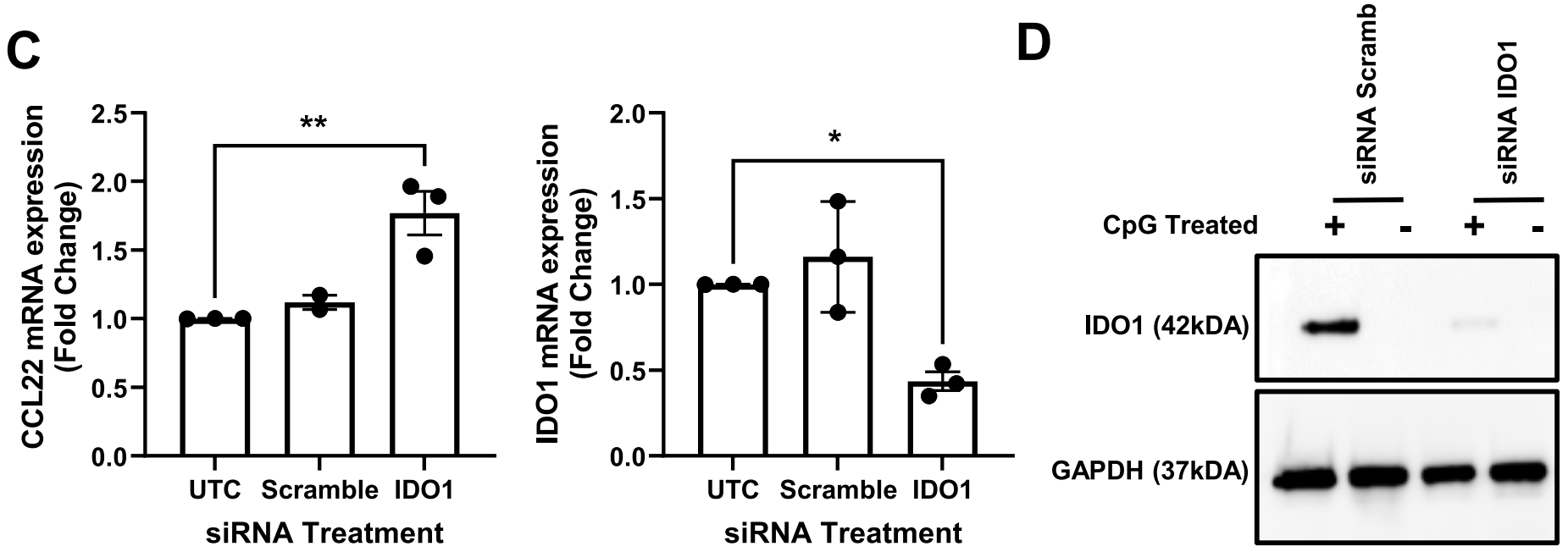

CpG
95 ASO
20 ASO
18 ASO
05 ASO
12 ASO
38 ASO
IDO1 (% control)
CCL22 (% control)
CCL22
IDO1
UTC

Results

The Kynurenine pathway is activated in cells treated with PS ASOs


A
Timepoint | Concentration
24 hrs | 8μM
24 hrs | 1.6μM
24 hrs | 0.32μM
48 hrs | 8μM
48 hrs | 1.6μM
48 hrs | 0.32μM
Tryptophan detected in THP1-TLR9 Cells
Tryptophan (umol/L)
0
10
20
30
40
50
CpG 5 18 95 12 20 38 UTC
ASO Treatment


B
Timepoint | Concentration
24 hrs | 8μM
24 hrs | 1.6μM
24 hrs | 0.32μM
48 hrs | 8μM
48 hrs | 1.6μM
48 hrs | 0.32μM
Kynurenine detected in THP1-TLR9 Cells
Kynurenine (umol/L)
0
2500
5000
7500
10000
12500
15000
CpG 5 18 95 12 20 38 UTC
ASO Treatment


C
CCL22 mRNA expression (Fold Change)
2.5
2.0
1.5
1.0
0.5
0.0
**
UTC
Scramble
IDO1
siRNA Treatment
IDO1 mRNA expression (Fold Change)
2.0
1.5
1.0
0.5
0.0
*
UTC
Scramble
IDO1
siRNA Treatment
D
siRNA Scramb
siRNA IDO1
CpG Treated
+
-
+
-
IDO1 (42kDA)
GAPDH (37kDA)

Conclusion

- IDO1 and IL4I1 regulate PS-ASO-induced innate immunity via the kynurenine pathway
- KP metabolites suppress excessive TLR9 activation
- Potential for co-therapy or formulation excipients to enhance tolerability
- Mechanistic foundation for future translational studies

Acknowledgements

Adam J Pollak for his critical and ongoing support.

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Thank you

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