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In Vitro Evaluation of Immunomodulating Drugs as Perpetrators of Drug Interactions

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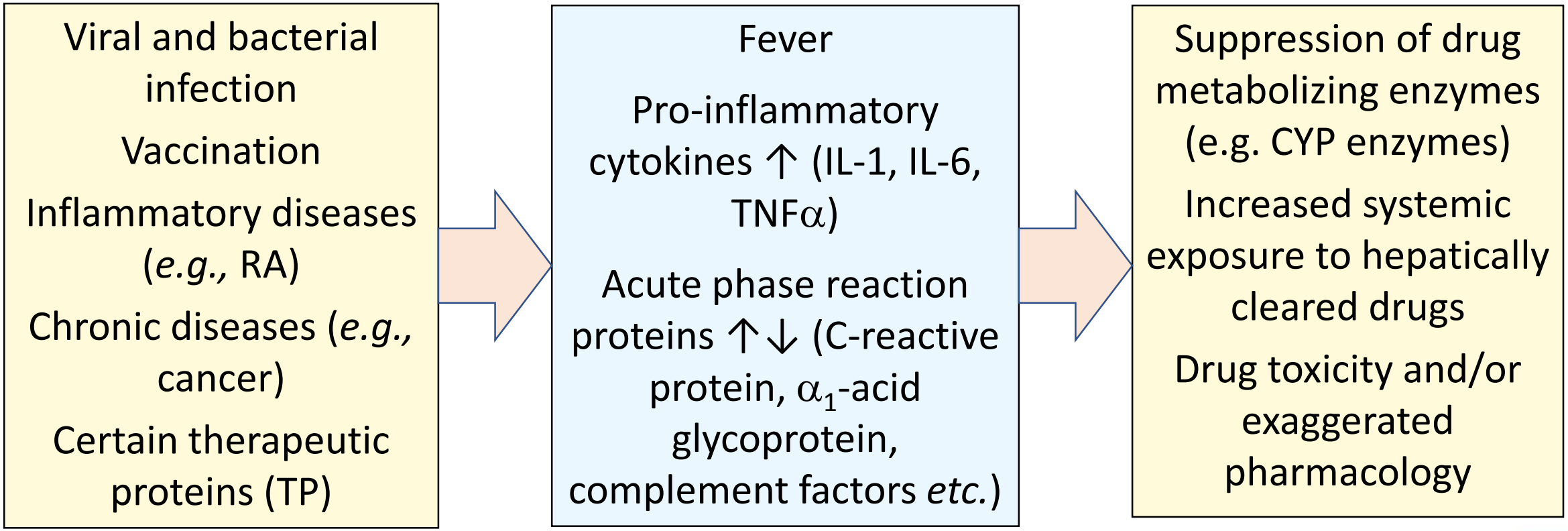
Presentation outline

- Immunomodulators
- Immunomodulator drug-drug interactions
- Regulatory guidance
- XenoTech study design
- Case studies

Immunomodulators

- Definition – immunomodulators are drugs that adjust immune response
  - Large molecules
    - antibodies, recombinant cytokines, cytokine receptors, vaccines
  - Small molecules
    - Tofacitinib, JAK inhibitor, in management of rheumatoid arthritis
    - Disease modifying drugs in multiple sclerosis such as azathioprine, cladribine, cyclophosphamide, methotrexate, and mitoxantrone
    - Thalidomide analogs in multiple melanoma
- Our emphasis is on compounds affecting cytokines

Immunomodulator drug-drug interactions



Immunomodulator drug-drug interactions

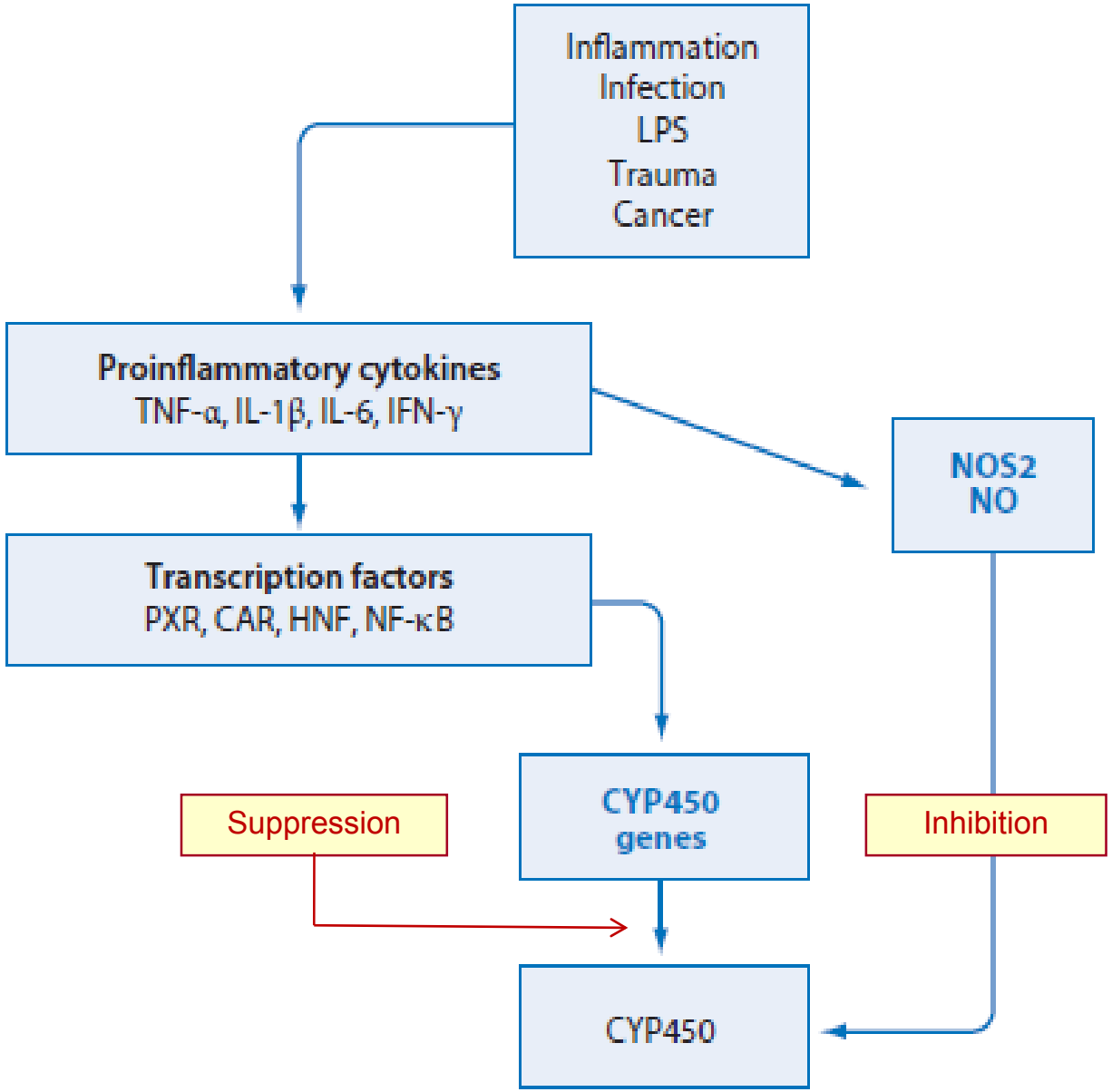
The ability of vaccination, cytokines (interferons, interleukins) to cause clinically significant impairment of the CYP-dependent metabolism of drugs has been know for decades

Some cytokines suppress CYP levels by acting **directly** on the hepatocyte. Examples: IL-1β, IL-6 and TNFα, **but not** others such as IL-2

Others suppress CYP levels **indirectly** by first causing the release of cytokines from Kupffer cells or peripheral blood mononuclear cells (PBMC. Examples: endotoxin (lipopolysaccharide or LPS, bacterial oligos that contain pathogen-associated molecular patterns e.g. CpG)

Cytokines can suppress CYP levels by **activating** certain transcription factors (such as NFκB, STAT1 and C/EBP-β) which diminish the transcriptional activity of other nuclear receptors such as CAR, PXR, GR and HNFs

Diminished drug metabolism during inflammation



Zhou and Mascelli (2011)
Annu Rev Pharmacol Toxicol **51**:539-572

Regulatory guidance on evaluating DDI potential of biologics

- FDA's 2012 guidance
  - Low potential for small molecule drugs to affect "therapeutic proteins" – some exceptions
  - Focus was on TPs as: cytokines, or peptide hormones that are *cytokine modulators* causing CYP suppression
  - Also covered was de-suppression (e.g., tocilizumab)
  - Noted "In vitro or animal studies have limited value in the . . . projection of clinical interactions"
    - Suggested clinical cocktail studies

Regulatory guidance on evaluating DDI potential of biologics

- Other scenarios mentioned in 2012 FDA guidance
  - TPs used in combination therapy – "evaluate effect of each product on the other"
  - "When there are *known* mechanisms or *prior experience* with certain PK or PD interactions, appropriate in vitro or in vivo assessments" should be conducted
    - Immunosuppressant effect of methotrexate – can alter antibody formation against a TP

Regulatory guidance on evaluating DDI potential of biologics

- Europe EMA 2013: Guideline on the investigation of drug interactions
  - "Interactions with therapeutic proteins including peptides . . .

    are not discussed in this guideline" – Refers to 2007 guideline
- EMA 2007: Guideline on clinical PK of TPs
  - "some therapeutic proteins (e.g. immunomodulators such as cytokines) have shown a potential for inhibiting or inducing CYP-enzymes and thus the need for in vitro or in vivo studies should be considered on a case-by-case basis"
  - "we lack knowledge about suitable tools to explore such interactions"
- Japan PMDA 2017: Drug Interaction Guideline

Is new FDA guidance on DDI evaluation for TPs coming soon?

- FDA posted a request for information and comments in May of 2018:
  1. In what scenarios and for which classes of TPs should DDI assessment be performed?
  2. What types of assessments can be useful?
  3. What are the study design considerations?

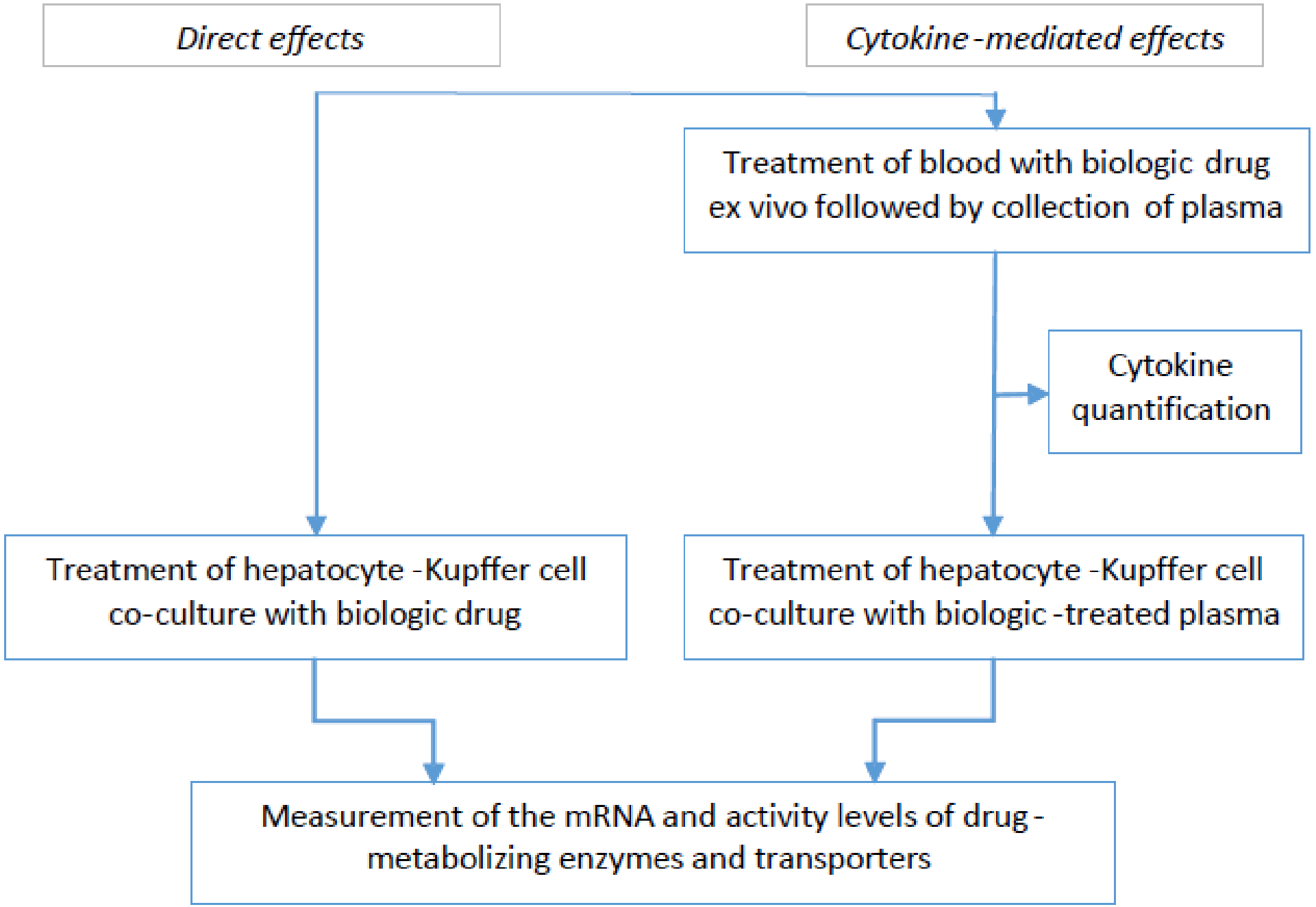
FDA Docket FDA-2018-N-1415

Regulatory guidance on evaluating DDI potential of biologics

- Comments were received by pharma companies (8), the IQ-consortium leadership groups (2), XenoTech
- Many recommendations, including:
  - Risk-based assessment, including patient population, etc.
  - FDA should state their position on the utility of in vitro assessments for TP DDI prediction
  - Restrict recommendations to TPs that modulate pro-inflammatory cytokines
  - Allow PopPK analysis of DDIs
  - In vitro studies with a TP should not be required when clinical translation has been previously established for a targeted TP (e.g., anti-IL6 mAb)

Regulatory guidance on evaluating DDI potential of biologics

- XenoTech (SXT) comments in the FDA docket:
  - In vitro testing in hepatocytes typically has been limited by:
    - Investigating a single recombinant cytokine at a time
    - Absence of other relevant cell types
  - SXT has developed “in vitro system to evaluate xenobiotics as immune-modulators of drug transport and metabolism in human hepatocytes”
    - Treatment of whole blood by TP followed by treatment of hepatocytes with the plasma allows for indirect effects to be evaluated.
    - Plasma from patients can be used in our studies


Direct effects
Cytokine-mediated effects
Treatment of blood with biologic drug ex vivo followed by collection of plasma
Cytokine quantification
Treatment of hepatocyte-Kupffer cell co-culture with biologic drug
Treatment of hepatocyte-Kupffer cell co-culture with biologic-treated plasma
Measurement of the mRNA and activity levels of drug-metabolizing enzymes and transporters

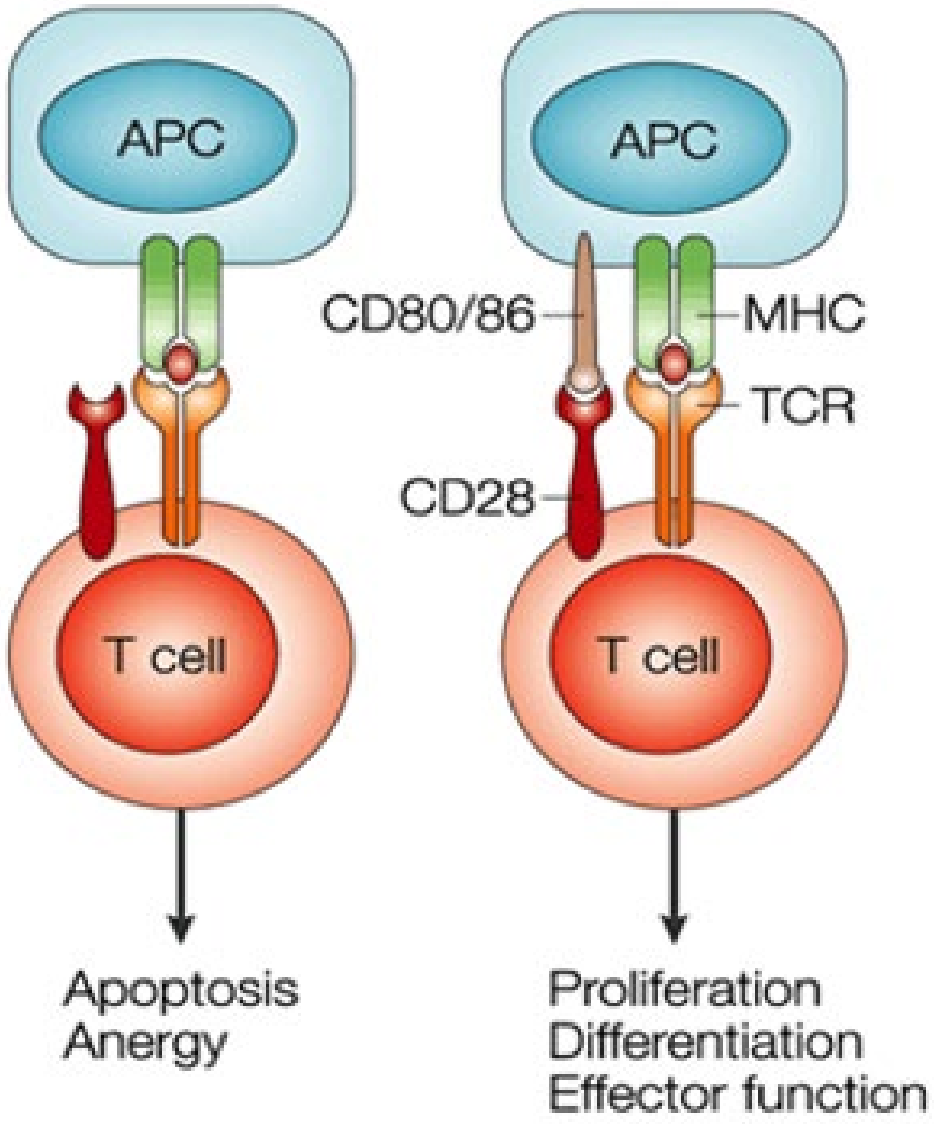
Case study - anti-CD28 mAb

Anti-CD28 monoclonal antibody-stimulated cytokines released from blood suppress CYP1A2, CYP2B6, and CYP3A4 in human hepatocytes in vitro

We chose ANC28 based on another anti-CD28 antibody, TGN1412, which caused cytokine storm and severe toxicity in a first-in-man study and dramatically highlighted the need to improve preclinical safety assessment of therapeutic proteins

Czerwiński et al., (2015) DMD **43**: 42-52

Role of CD28 in regulation of T cells



Nature Reviews | Immunology

CD28 mAb case – scope of the study

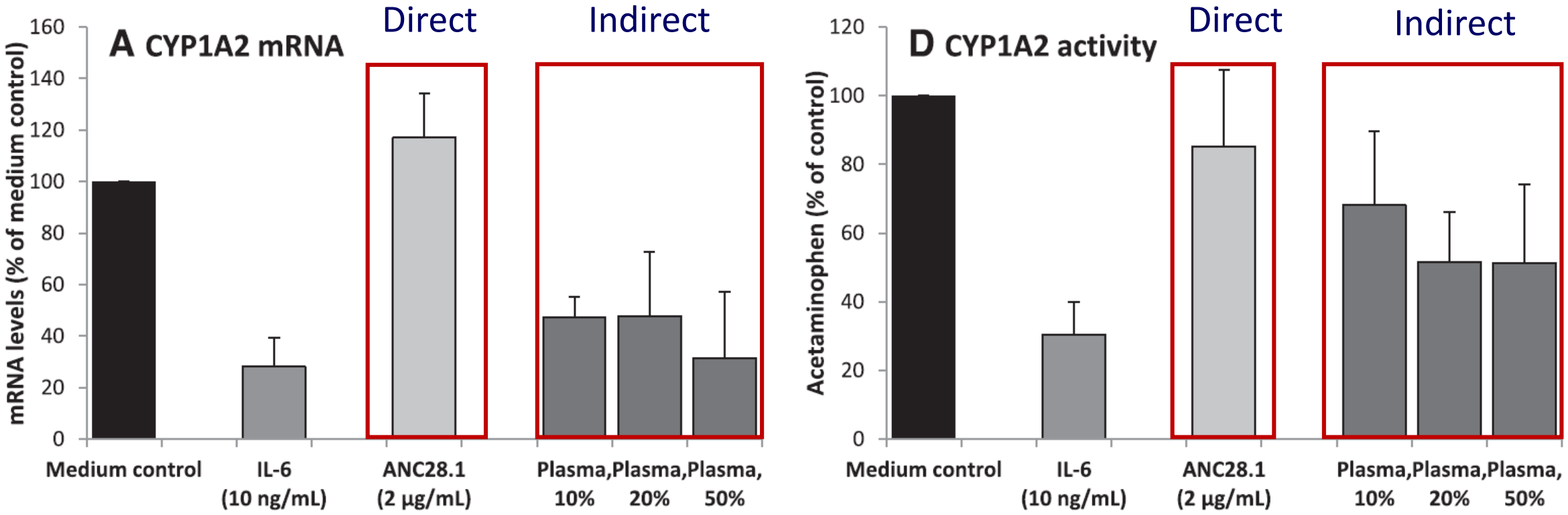
Parameter	
Blood treatments	4 donors, 24 h, 37°C, one concentration of the mAb
Cytokines analyzed in plasma	9
Number of hepatocyte co-cultures	3
Plasma in cell culture medium	10%, 20%, 50% v/v
Analysis end-points	mRNA and activity of CYP1A2, 2B6, 3A4

CD28 mAb – cytokine release

Cytokine	Treatment			
	Saline	LPS	MOPC antibody	ANC28 antibody
Interferon-ɣ	4.7	5160	2.1	109
Interleukin-1β	2.3	5520	0.53	18.1
Interleukin-2	8.2	107	8.0	749
Interleukin-6	4.7	12000	1.2	110
Interleukin-8	800	10000	490	5400
Interleukin-12	7.2	55.0	2.6	BLQ
TNF-α	8.5	5830	5.7	227
GM-CSF	15	200	21	540

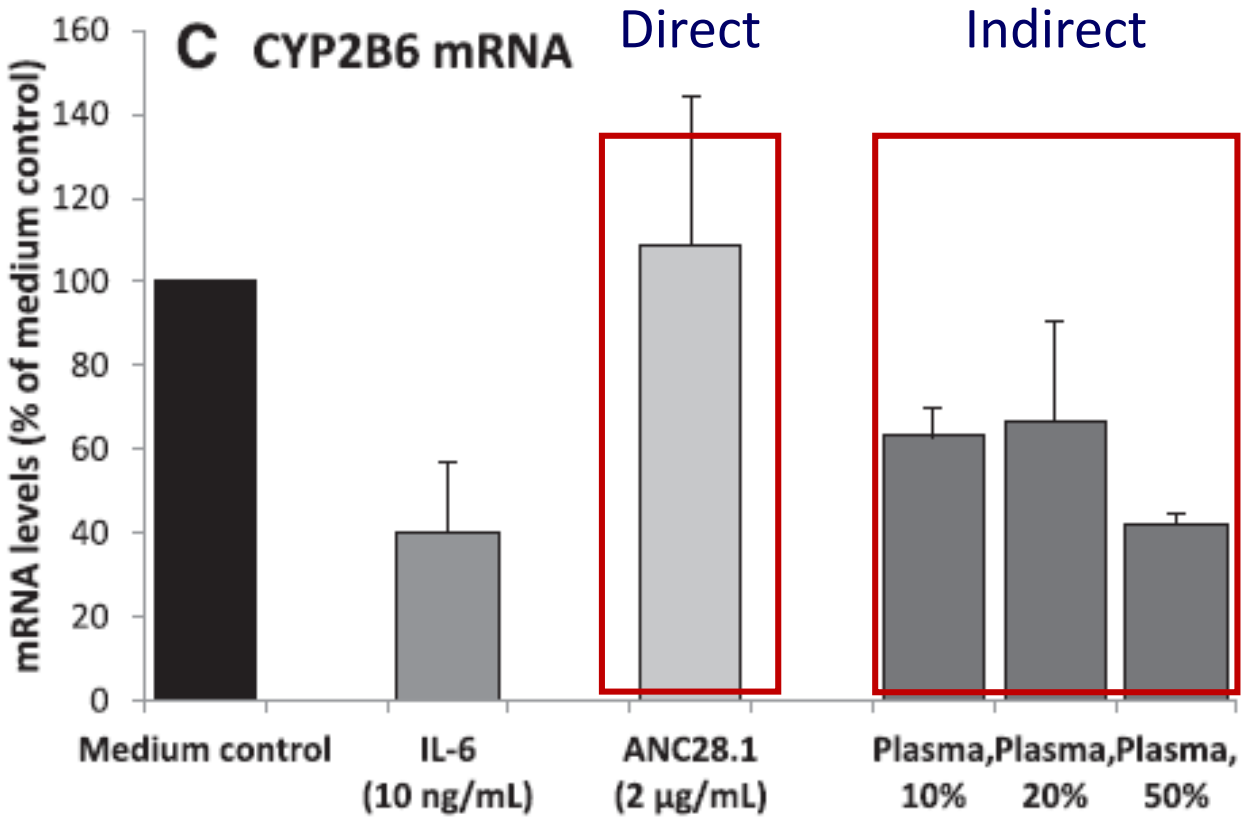
ANC28 caused significant increases in IFN-γ, IL-2, IL-6, IL-8, TNF-α and GM-CSF

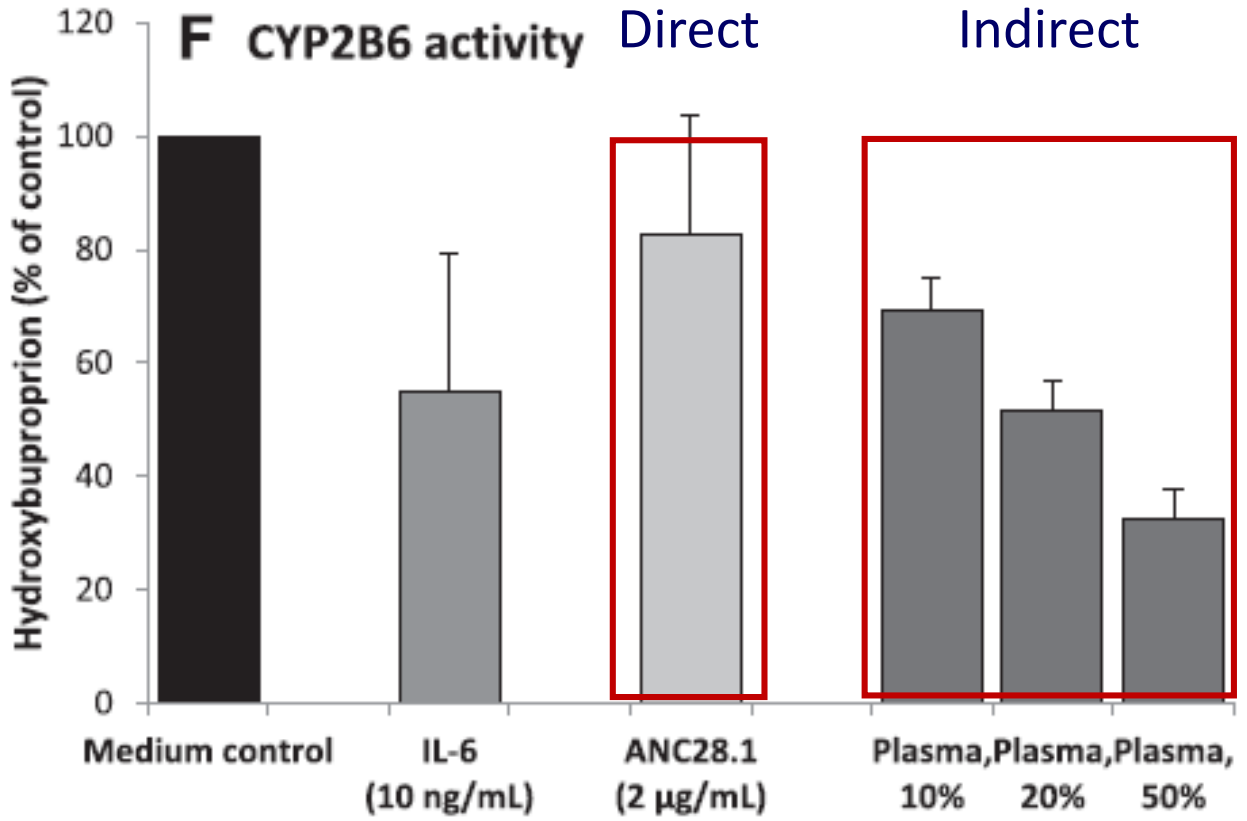
CD28 mAb – indirect suppression of CYP1A2



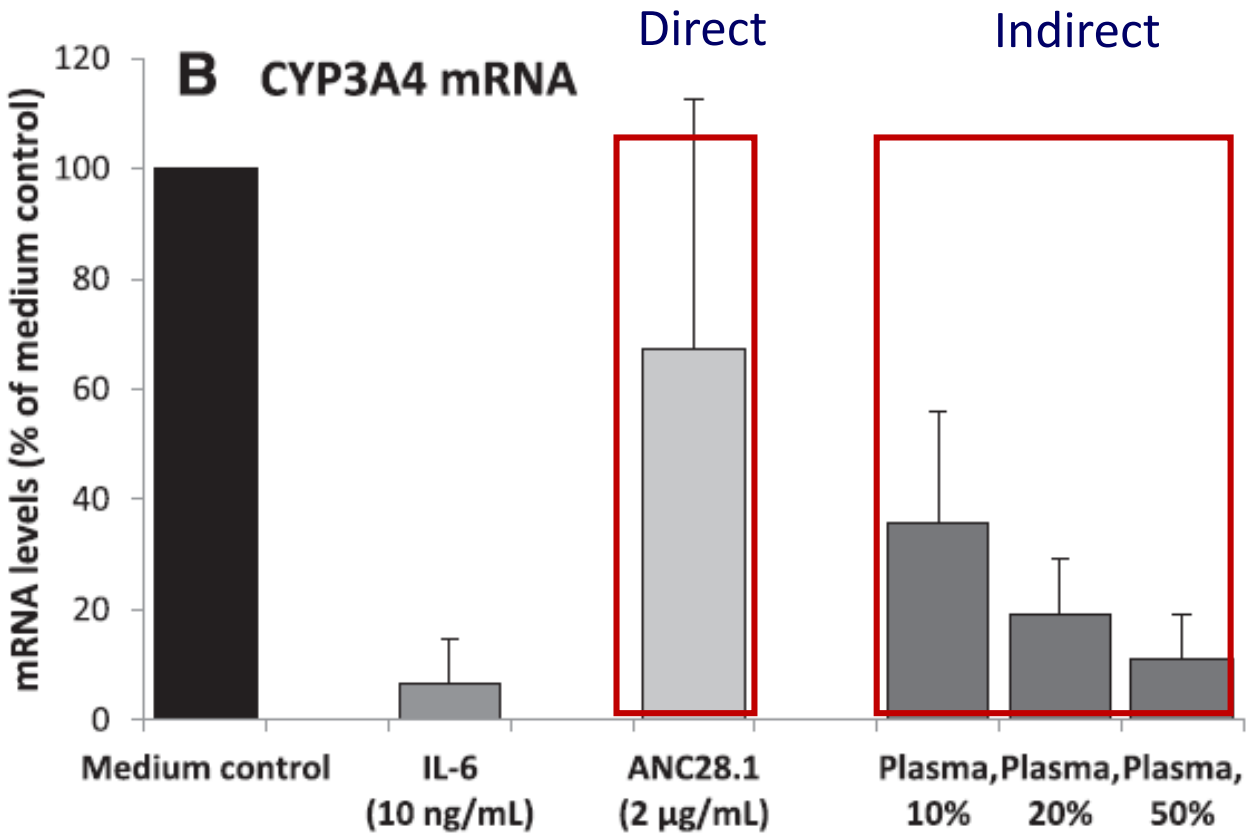
Primary cultures of human hepatocytes from three donors were incubated for 72 hours with medium alone, medium containing IL-6, ANC28.1, or plasma prepared from blood treated with ANC28.1

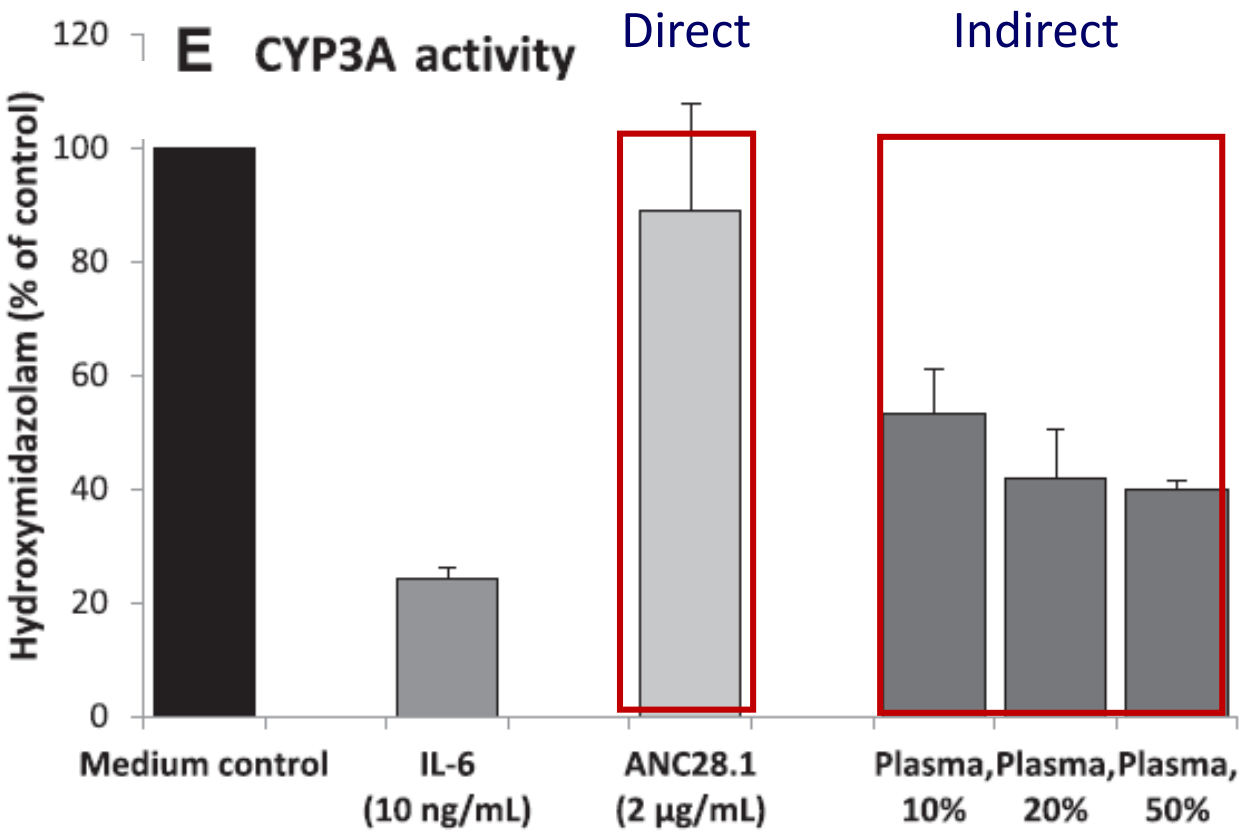
CD28 mAb – indirect suppression of CYP2B6





CD28 mAb – indirect suppression of CYP3A4





CD28 mAb – conclusions

- LPS and ANC28 antibody caused the release of multiple cytokines from whole human blood ex vivo
- Plurality of the cytokines suppressed the mRNA and the enzymatic activity of hepatic CYPs in vitro while the Ab itself did not
- DDI potential of certain therapeutic proteins, such as ANC28, can be identified in vitro provided that cytokine release in blood is taken into account

Case study - albumin-fused growth hormone TV-1106

Direct and cytokine-mediated effects of albumin-fused growth hormone, TV-1106, on CYP enzymes expression in human hepatocytes in vitro

TV-1106: human growth hormone genetically fused to human albumin. Fusion of GH and A—a carrier protein without hormone activity, but with a long plasma half-life, extends systemic circulation of GH and preserves hormone activity. TV-1106 is being developed for treatment of GH deficiency to provide a sustained exposure.

Czerwiński et al., (2018) PRP **6**: 42-52

TV-1106 – cytokine release

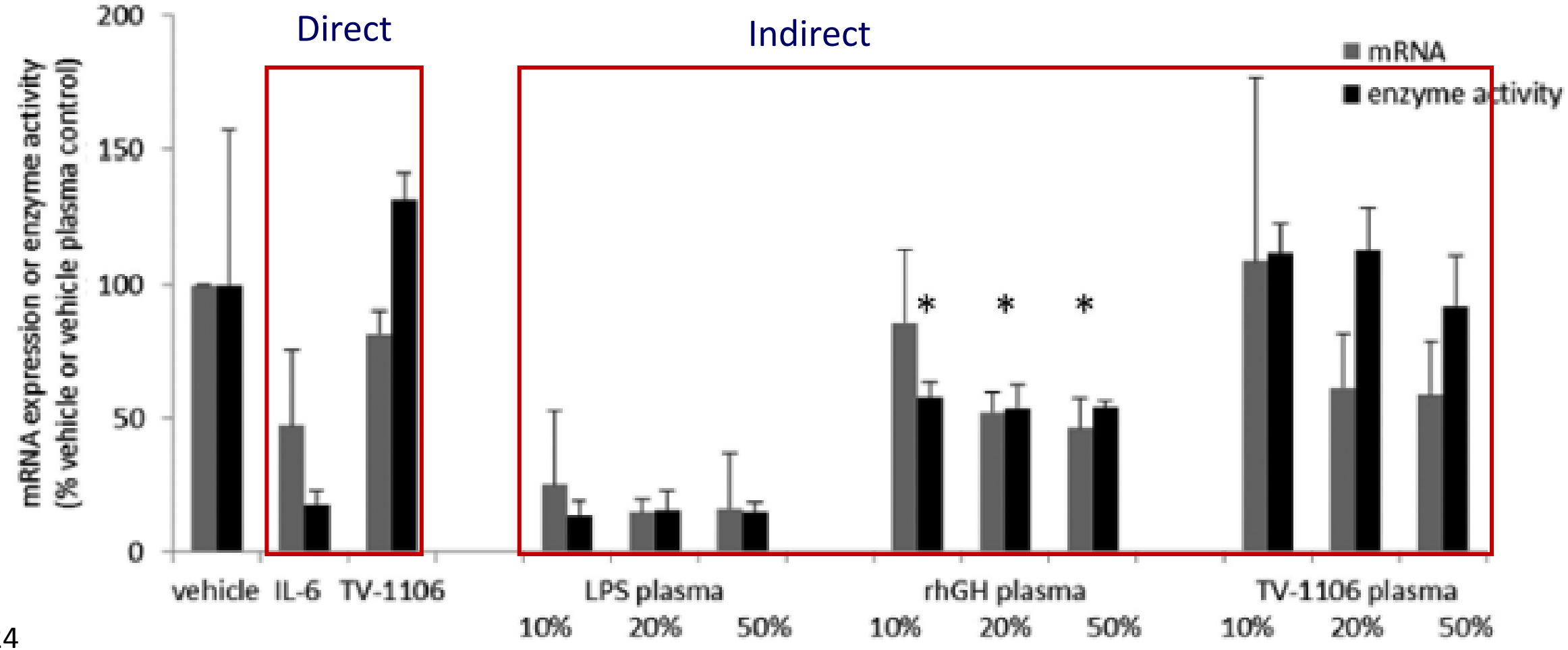
The effects of drug vehicle, LPS, GH or TV-1106 on cytokine release in whole blood ex vivo

Cytokine	Treatment			
	Saline	LPS	Growth hormone	TV-1106
Interferone-ɣ	15	3000	40	7.7
Interleukin-1β	4.6	13000	90	20
Interleukin-2	33	320	213	61
Interleukin-6	32	15000	880	78
Interleukin-8	2500	11000	5800	3500
Interleukin-10	8.8	1200	28	5.1
Interleukin-12p70	24	46	5.5	13
TNF-α	20	8500	130	27
GM-CSF	82	470	306	230

TV-1106, on an equimolar basis for the hormone, caused release of smaller amounts of cytokines than unmodified GH. IL6 increased by GH was significantly higher than the cytokine in the TV-1106 plasma

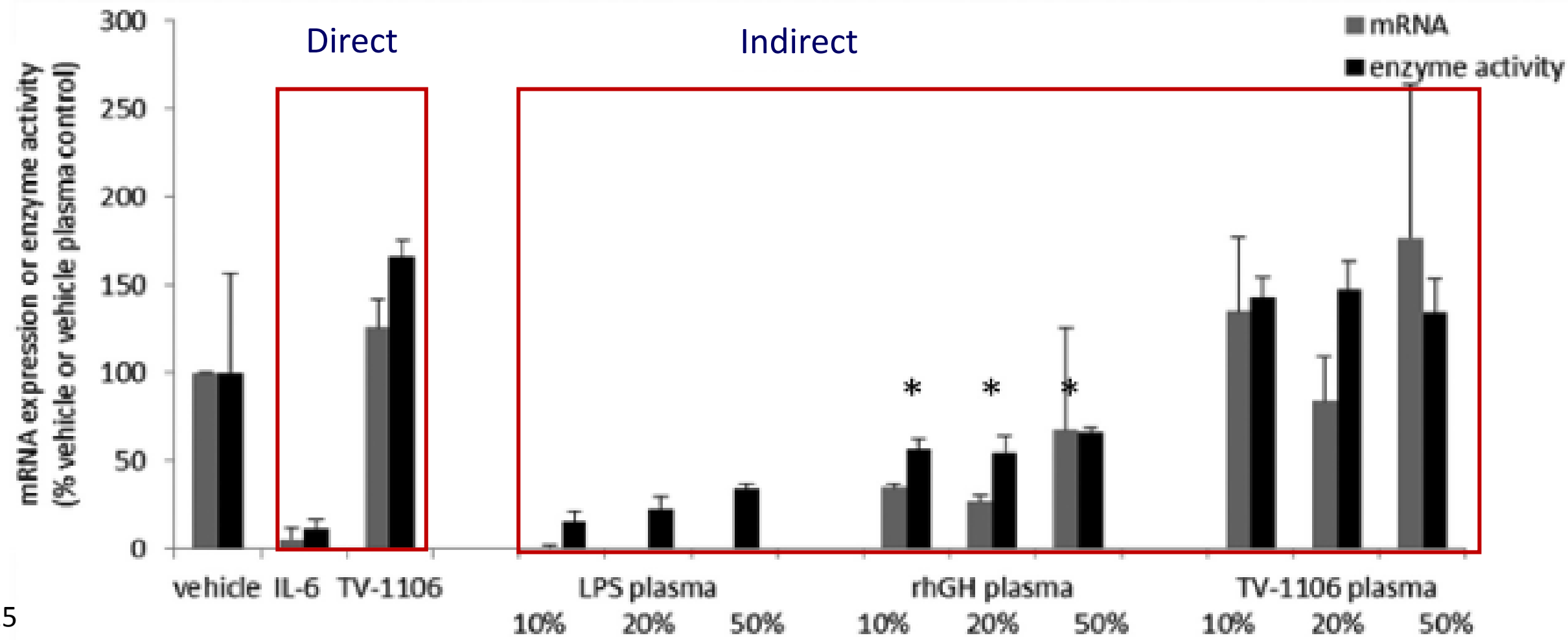
TV-1106 – effects on CYP1A2

Direct and indirect effects of GH and TV-1106 on CYP1A2



TV-1106 – effects on CYP3A4

Direct and Indirect effects of GH and TV-1106 on CYP3A4



TV-1106 - conclusions

- Treatment of blood with GH increased multiple cytokines, while treatment of blood with TV-1106 had no effect on the nine cytokines
- GH had little or no direct effect on CYP1A2 or CYP2C19 mRNA but increased CYP3A4 mRNA twofold (not shown), the GH indirectly suppressed CYP1A2 and CYP3A4
- TV-1106 had little or no, direct or indirect effect on CYPs mRNA or activity
- In contrast to GH, albumin-fused TV-1106 was unlikely perpetrator of CYP1A2, 2C19 or 3A4- direct or cytokine-mediated DDI

Case study - Tilsotolimod

Cytokine-mediated suppression of CYP enzymes by the Toll-like receptor 9 agonist, Tilsotolimod, in cultured human hepatocytes

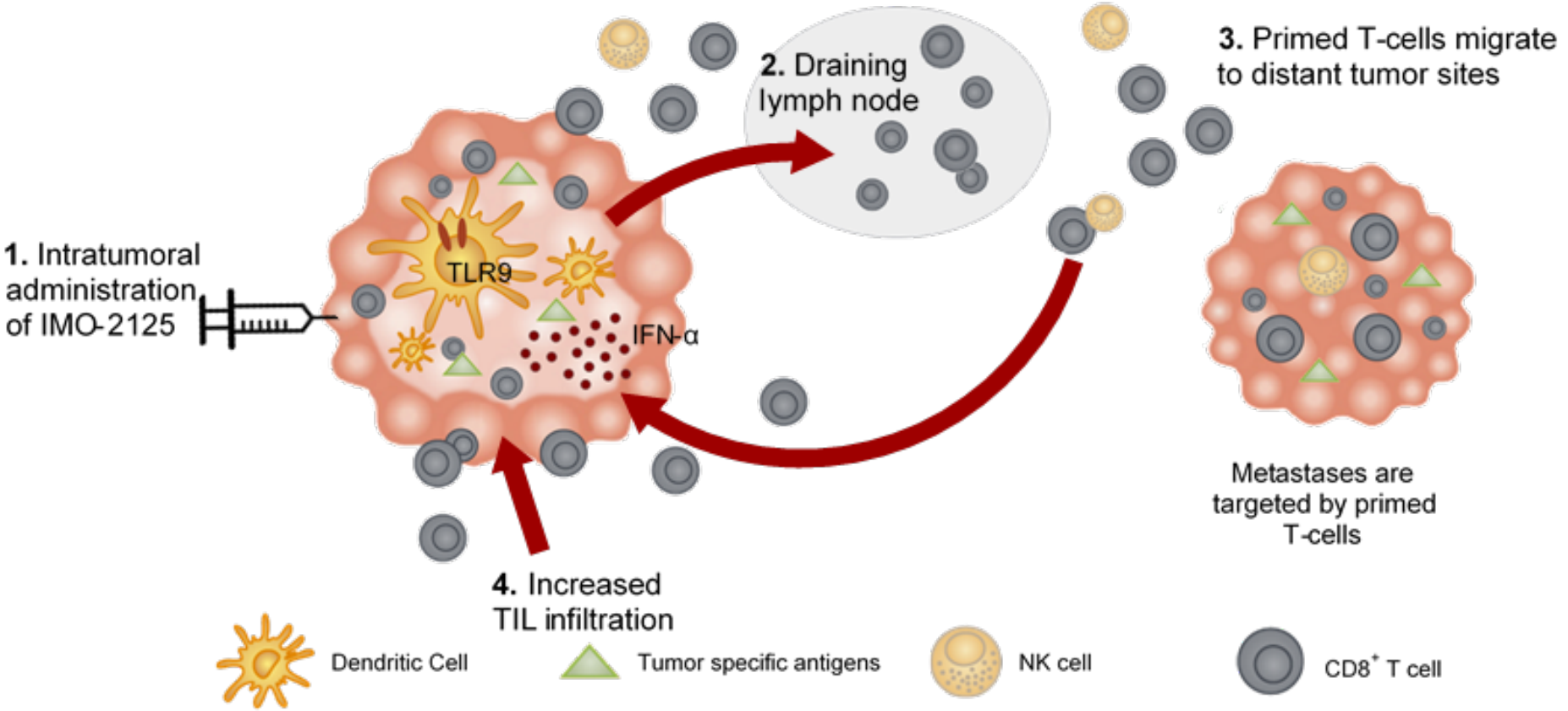
Paul Tarantino, Tim Sullivan, Brian W. Ogilvie, Maciej Czerwiński

Idera Pharmaceuticals, Inc., XenoTech LLC

Although not a peptide drug, it is possible that an immuno-modulator, such as the oligonucleotide, Tilsotolimod, an agonist of toll-like receptor 9 designed to enhance T-cell responses to tumor antigens, could precipitate drug-drug interactions.

International Journal of Toxicology 2019, Vol. 38(1) 61, P511

Tilsotolimod – mechanism of action

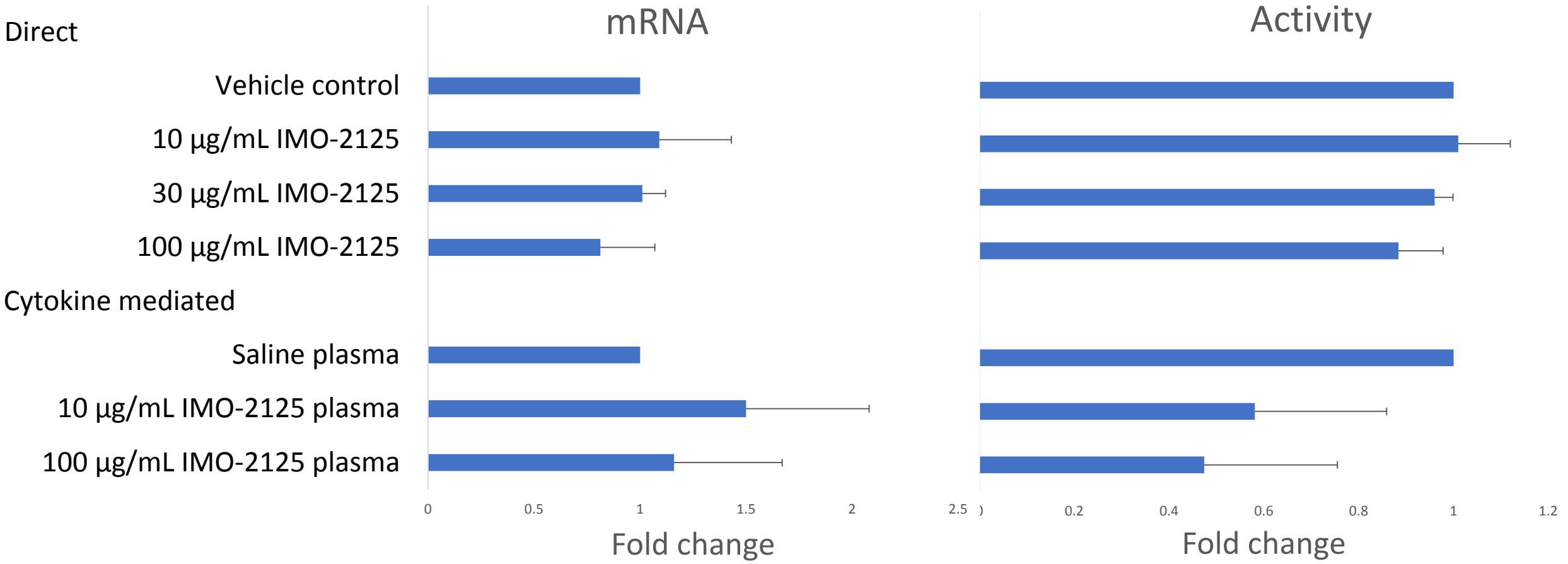


Tilsotolimod – cytokine release

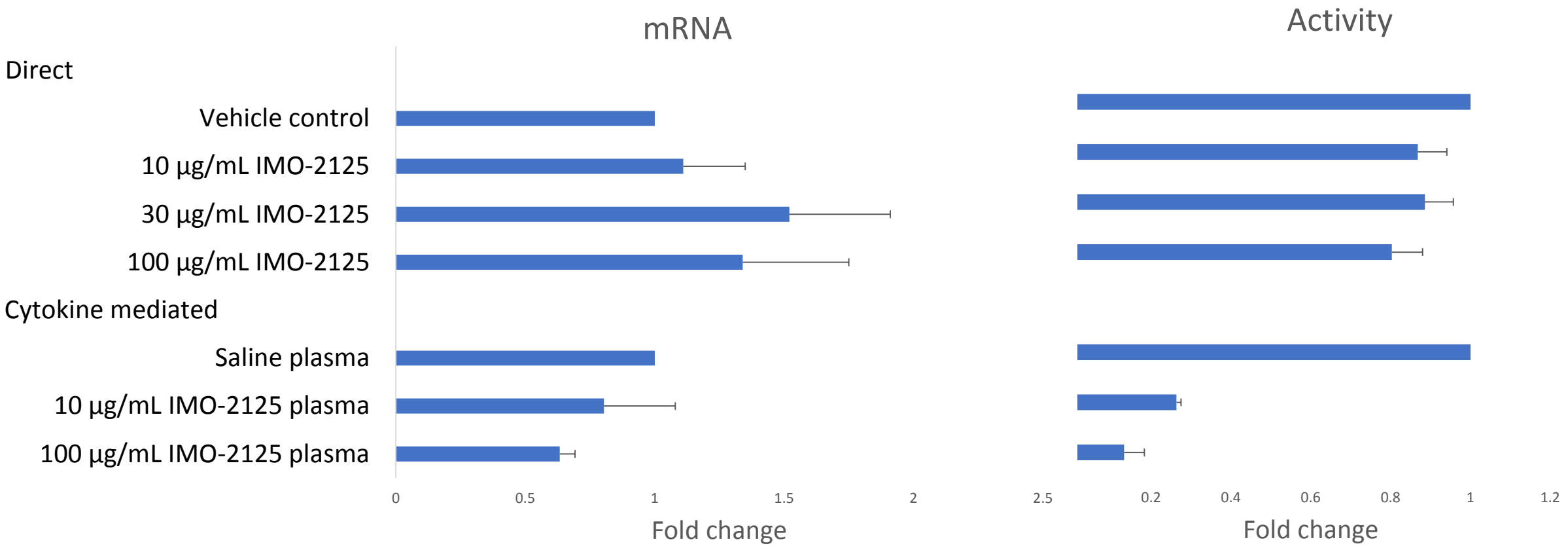
Cytokine pg/mL	Treatment			
	Saline	LPS	Tilsotolimod 10 µg/mL	Tilsotolimod 100 µg/mL
IP-10	350	25000*	22000	5700*
MCP-1	6800	1600*	11000	12000*
MIP-1α	730	ALQ	990	2500*
Interleukin-2	0.33	1.2*	0.37	0.42
Interleukin-6	140	8400*	160	64
Interleukin-12p70	2.7	74*	2.7	2.0
TNF-α	16	3600*	20	43*
IFN-α2a	BLQ	0.33	170	490*

*Statistically different from saline control

Tilsotolimod – suppression of CYP1A2 activity



Tilsotolimod – suppression of CYP2B6 activity



Tilsotolimod - conclusions

- Agonists of Toll-like receptors 4 and 9 produce different cytokine stimulation profiles
- Tilsotolimod suppressed CYP1A2 and CYP2B6 enzyme activities in a cytokine-mediated way. Drug did not have direct effects on CYP1A2, 2B6 or 3A4

Overall conclusions

- Cytokine release by PBMCs, Kupffer cells, etc. should be accounted for and XenoTech method incorporates the effects of these molecules
- Presented cases have demonstrated utility of the in vitro study design
- Current regulatory guidance is minimal – FDA solicited comments for a new guidance document

Thank You

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