

# Nonclinical Toxicity Assessment of Oral Tobacco-Derived Nicotine Products: V. In Vitro Mechanistic Assays Using Human Gingival Fibroblasts

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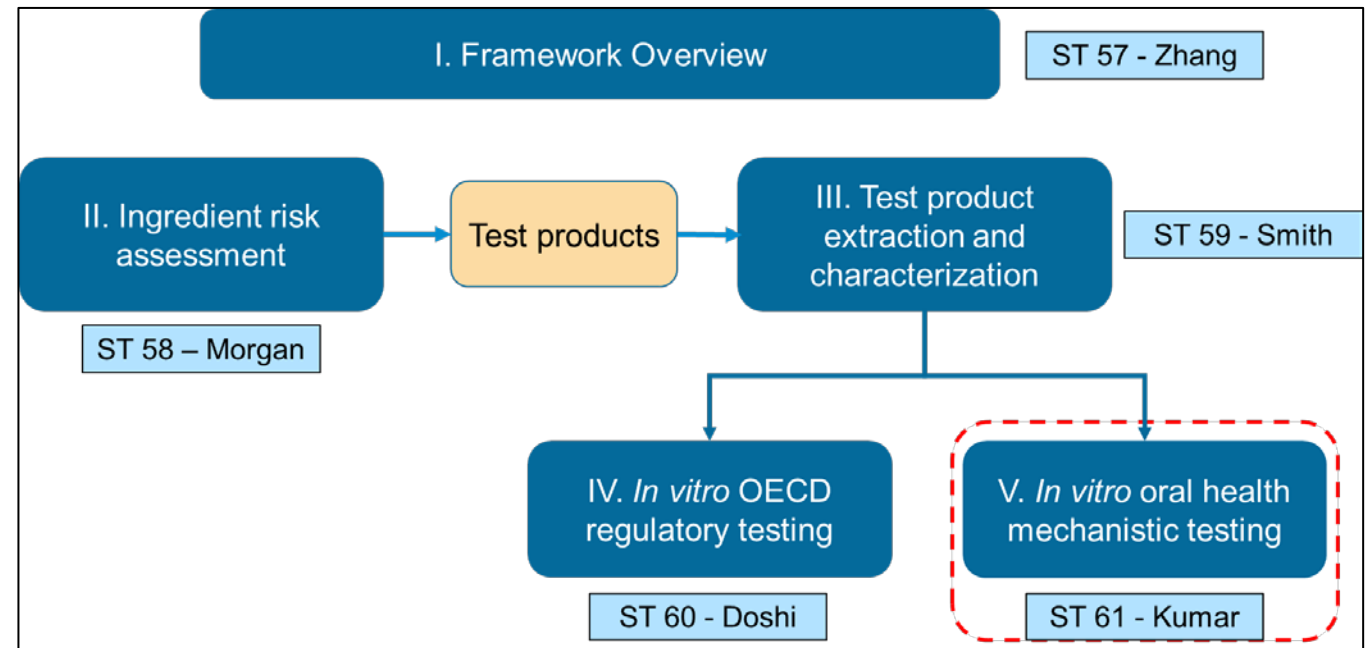
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Center for Research and Technology*

**CORESTA Smoke-Techno (SSPT) Conference - [ST 61]**

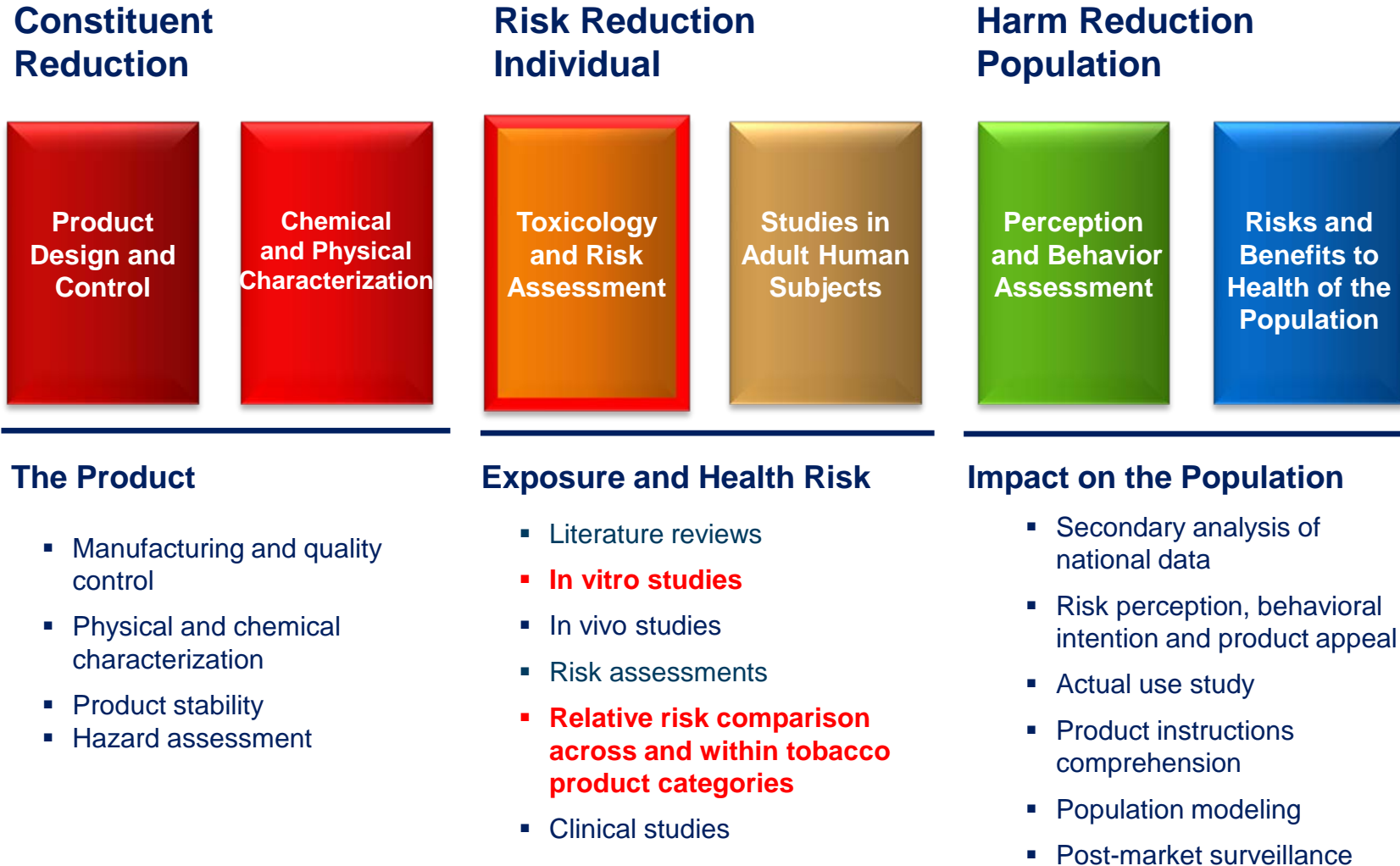


# Agenda

- Introduction
- Study Design
- Results
- Mechanistic Relevance of Oral Health *In Vitro* Endpoints
- Summary
- References



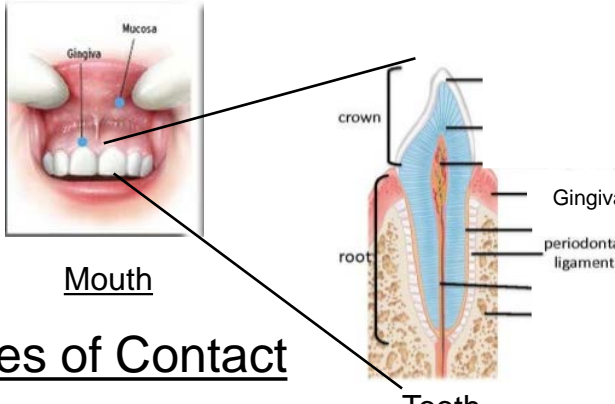
# Tobacco Harm Reduction Framework



# Introduction: Oral Health *In Vitro* Evaluation



**Product Placement**  
<https://www.onnicotine.com/>



**Sites of Contact**  
<https://www.jamesislanddentists.com/dental-health>

**Oral Epithelium**

- Primary
- Cell-line (TR146)

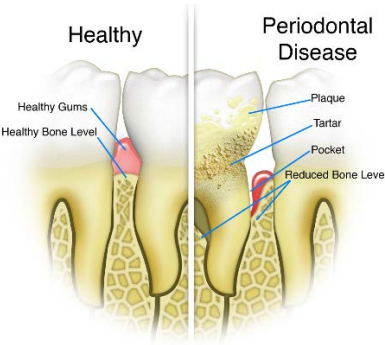
**Periodontal**

- Ligament Fibroblasts (PDL)

**Gingival**

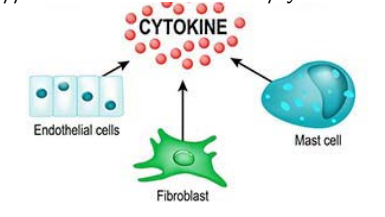
- Fibroblasts (HGF)

**Relevant In Vitro Models**

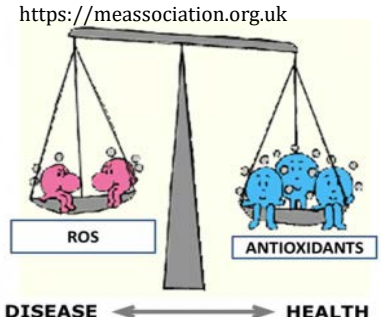


**Clinical Relevance**

**Mechanistic Toxicity**  
<http://www.biosciencenotes.com/cytokines>



**Inflammation**



**Oxidative Stress**  
<https://meassociation.org.uk>

Test Articles



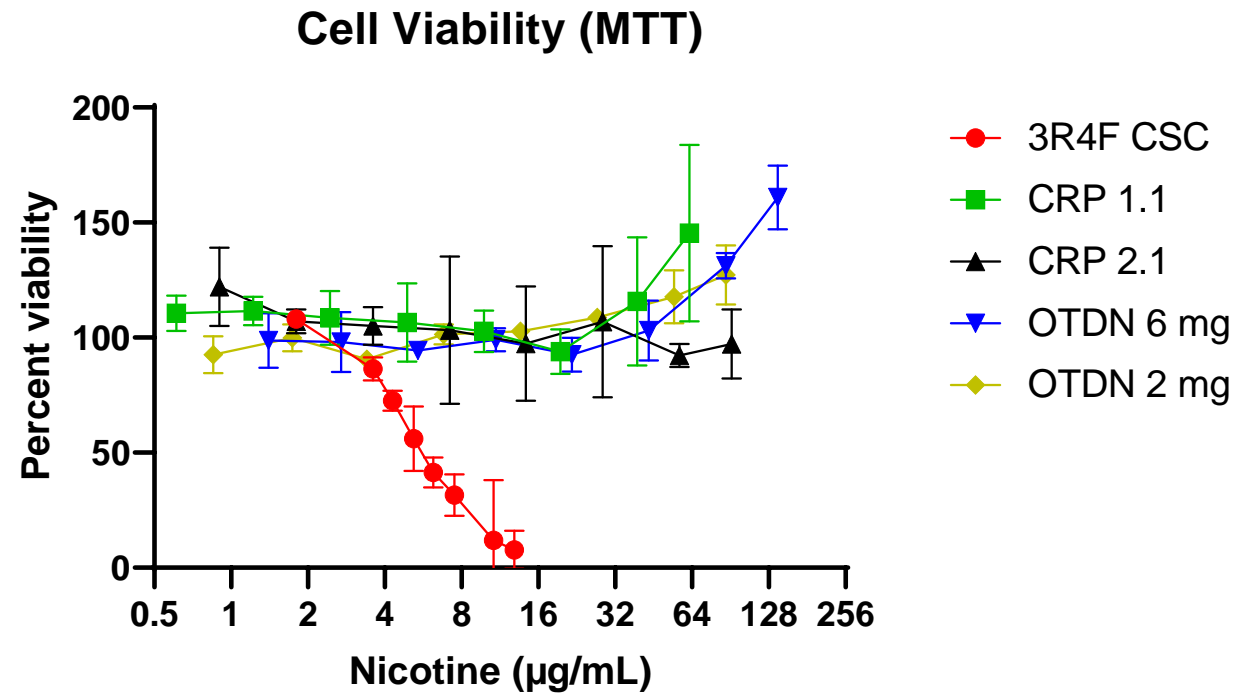
## ■ Feasibility testing (ethanol extracts)

- To evaluate test system (HGF cells), responding endpoints (cytotoxicity, oxidative stress and inflammation), and time of exposure for in vitro studies
  - Test articles: 3R4F, CRP2.1 (reference MST), CRP1.1 (reference Snus), and OTDN-6 mg (mint)
  - Maximum Dose: 1% v/v of extract
- Feasibility testing confirmed HGF cells as test system, responding endpoints (MTT, IL-6, IL-8, MMP-1, TIMP-1, PGE-2, GSH, and MDA) after 24 hours of exposure

## ■ Definitive testing (artificial saliva extracts)

- To characterize categorical differences in mechanistic endpoints using physiologically relevant matrix
  - Test articles: 3R4F, CRP2.1, CRP1.1, and OTDN-6 mg (mint), OTDN-2 mg (citrus)
  - Maximum Dose: extracts in artificial saliva 10 % v/v; 3R4F extracts in ethanol 1% v/v
  - Test System and exposure: HGF cells, 24 hours of exposure

# Results: Cytotoxicity

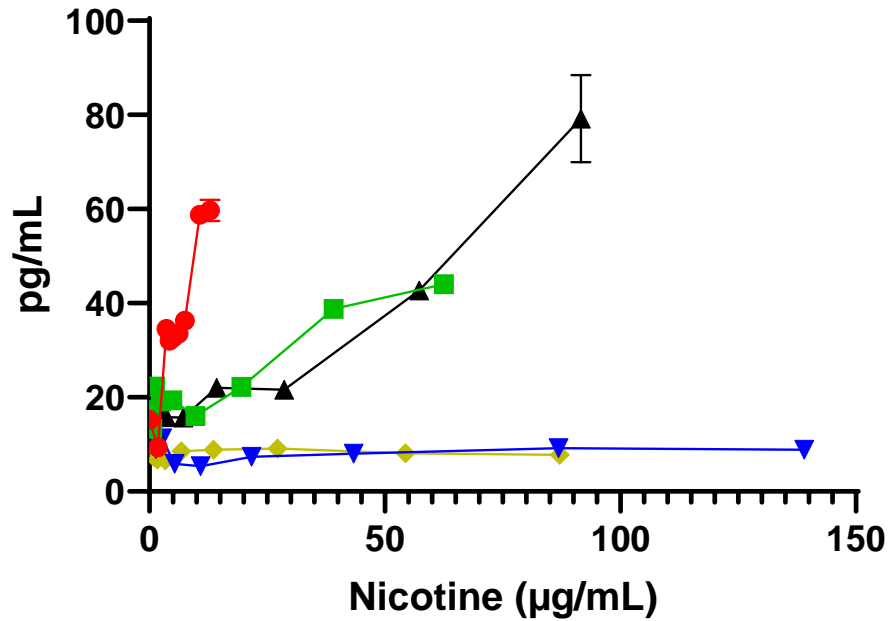


3R4F>>> Oral



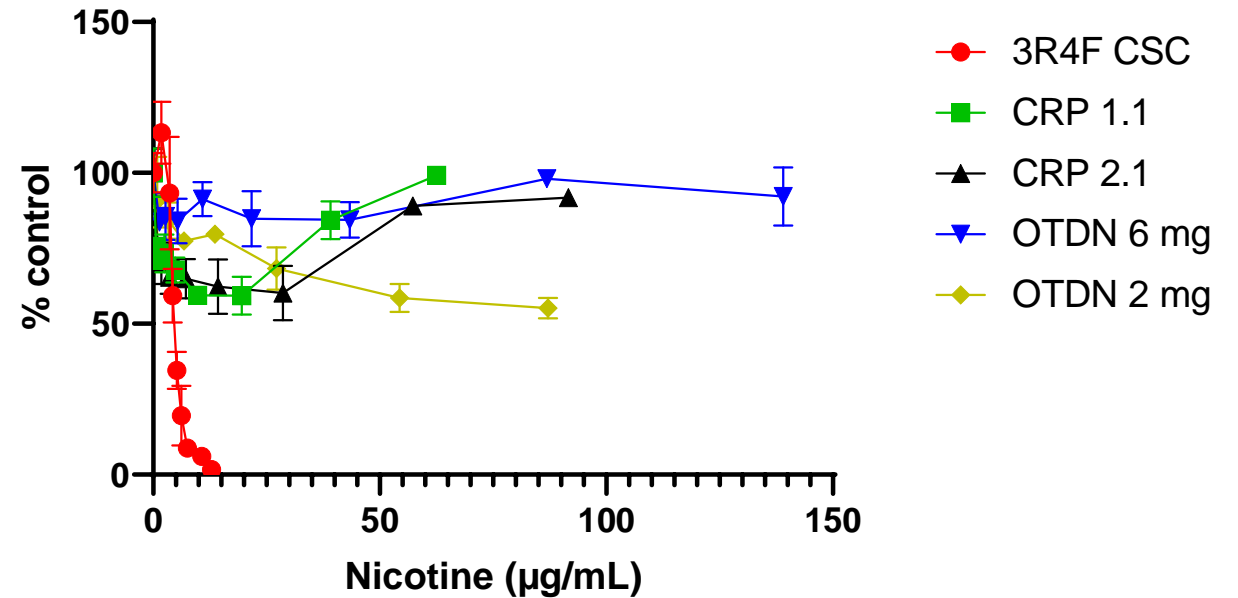
# Results: Oxidative Stress

### Lipid Peroxidation (MDA)



3R4F > CRP2.1 ~ CRP1.1 > OTDN\*

### Glutathione content (GSH)



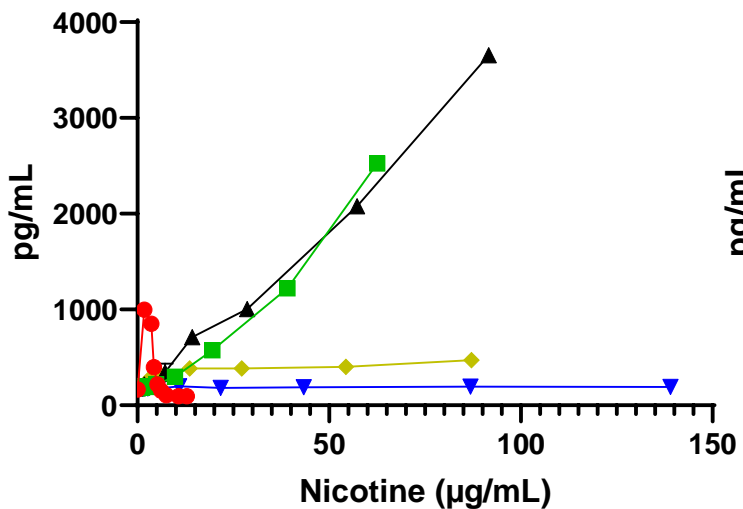
3R4F >> Oral

\* Based on tested OTDN products

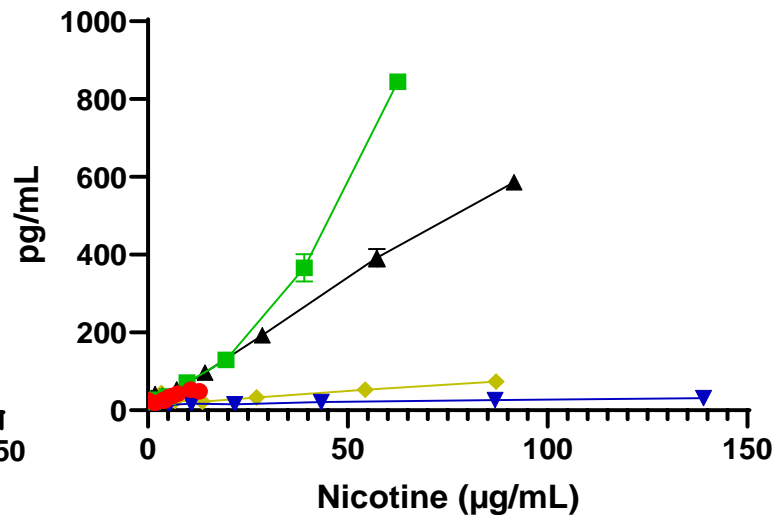


# Results: Inflammatory Mediators

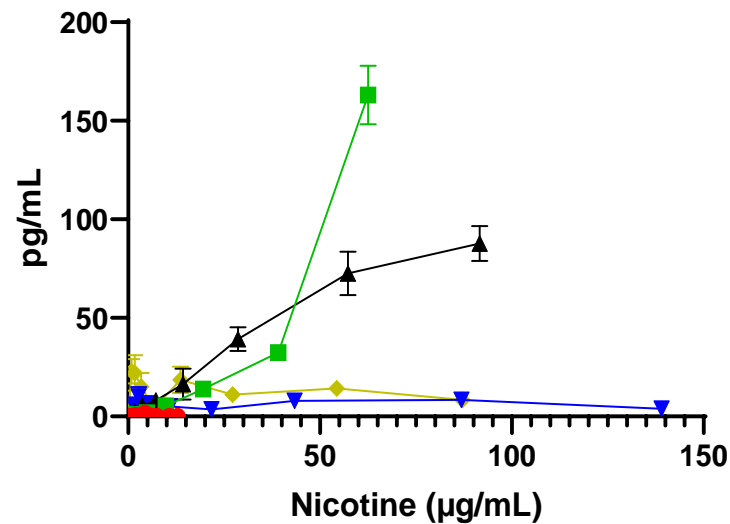
### Interleukin-8 (IL-8)



### Prostaglandin E2 (PGE-2)



### Interleukin-6 (IL-6)



- 3R4F CSC
- CRP 1.1
- ▲ CRP 2.1
- ▼ OTDN 6 mg
- ◆ OTDN 2 mg

CRP2.1~CRP1.1 > OTDN\*

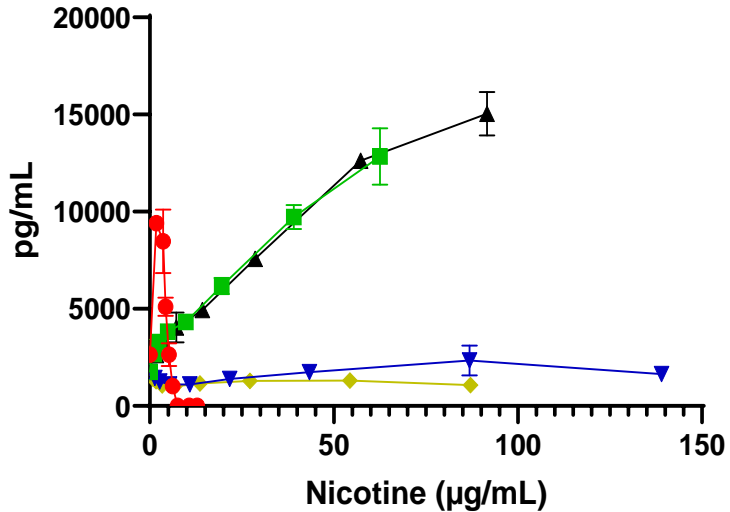
\* Based on tested OTDN products





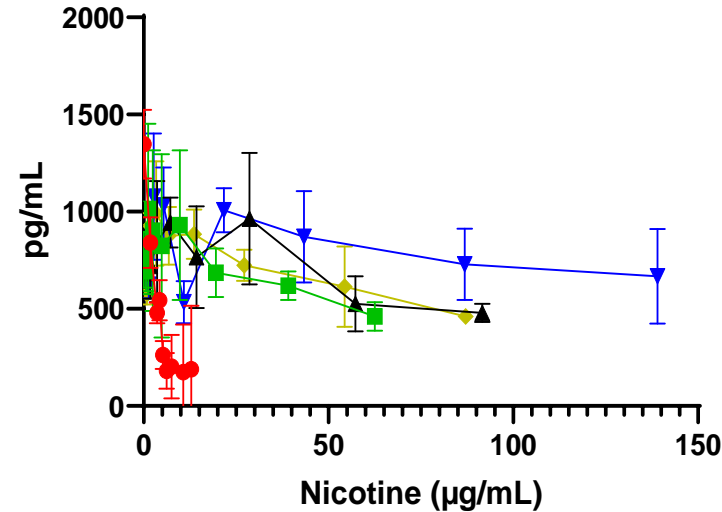
# Results: Inflammatory Mediators

Matrix Metalloproteinase-1 (MMP-1)



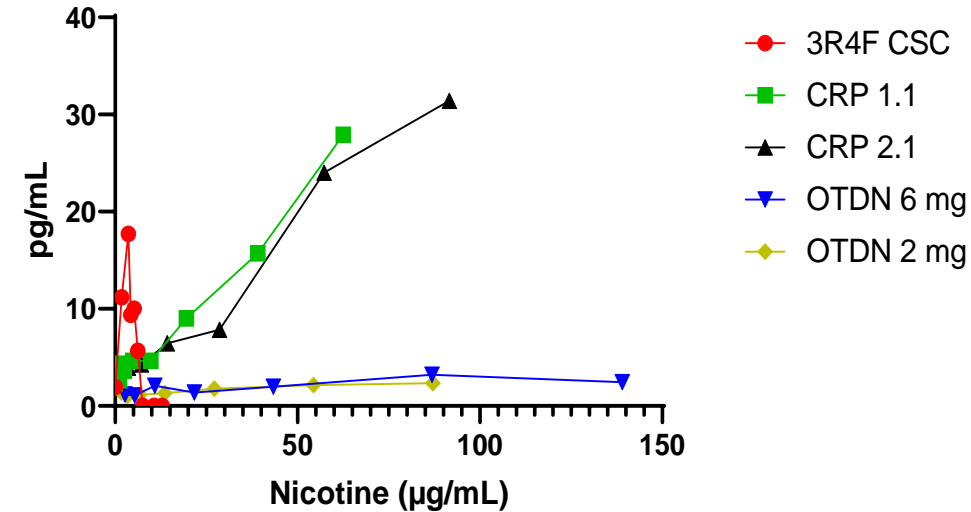
CRP2.1~CRP1.1 > OTDN\*

Tissue Inhibitor of metalloproteinase-1 (TIMP-1)



3R4F >> Oral

MMP-1/TIMP-1 ratio

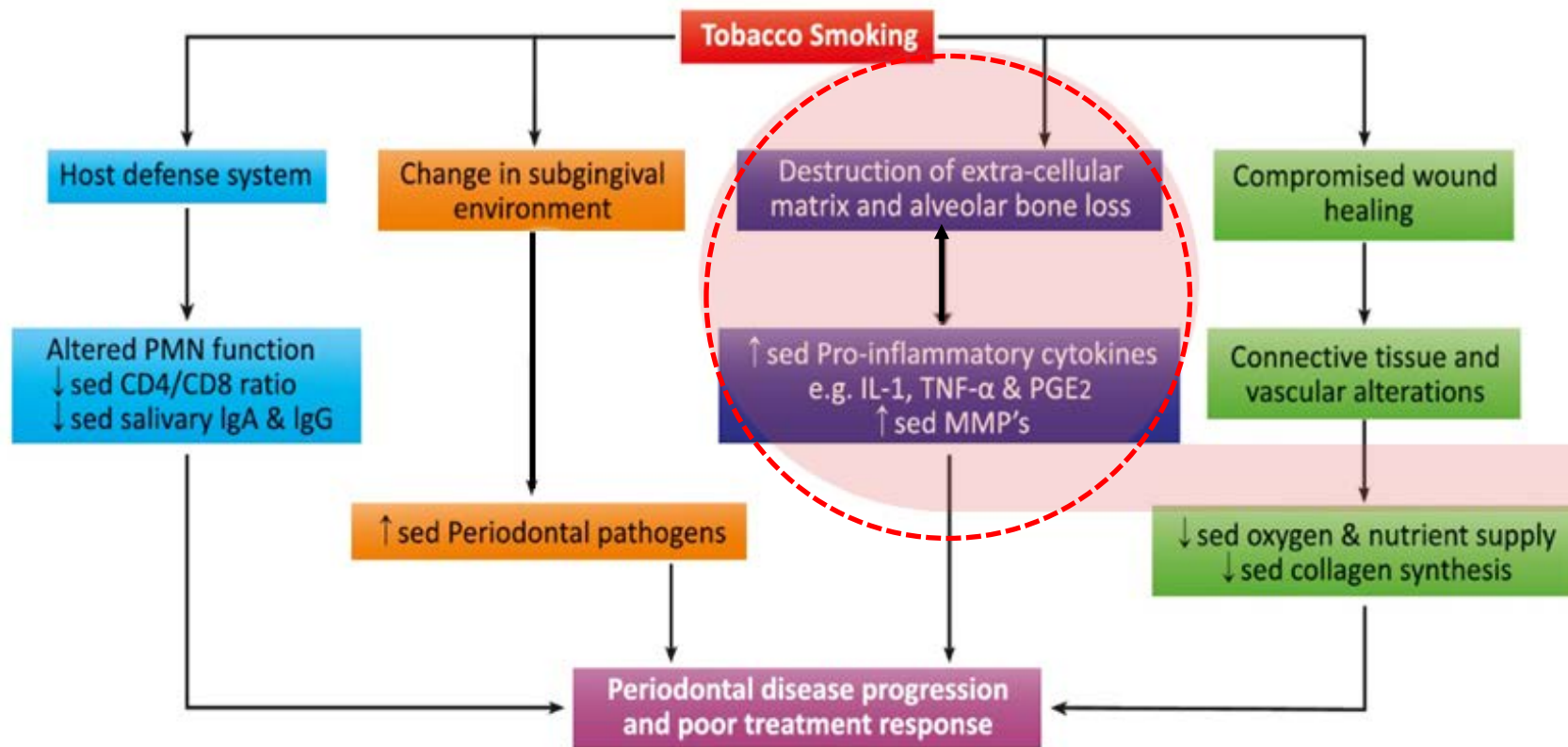


CRP2.1~CRP1.1 > OTDN\*

\* Based on tested OTDN products



# Mechanistic Relevance of Oral Health *In Vitro* Endpoints



Flowchart describing the effects of smoking on the host defence system, changes in the subgingival environment, and wound healing.



- Oral health *in vitro* mechanistic endpoints responded in HGF cells
- Results showed categorical differences in *in vitro* biological activities in human oral health (e.g., periodontal diseases) related mechanistic endpoints
  - 3R4F extracts showed cytotoxicity, increased oxidative stress and inflammation, whereas tested OTDN (2 market products) extracts showed no changes in these endpoints
- Based on the market products tested, results demonstrated substantially lower biological activity of OTDN products in comparison to cigarette smoke condensate





# References

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# Questions?

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