LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Integrated Data Analysis of *In Vitro* Testing Approaches Advancing BioTech Product Development and Safety.

Erwin L Roggen

Sens-it-iv Consortium

SIXTH FRAMEWORK PROGRAMME

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

A few clarifications

- 'My' BioTech products:
  - Industrial enzymes
  - Pharma proteins and peptides

SIXTH FRAMEWORK PROGRAMME

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

A few clarifications

- 'My' BioTech products:
  - Industrial enzymes
  - Pharma proteins and peptides

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

A few clarifications

- 'My' BioTech products:
  - Industrial enzymes
  - Pharma proteins and peptides
- 'My' Expertise:
  - Irritation
  - Carcinogensis
  - Sensitization

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

A few clarifications

- 'My' BioTech products:
  - Industrial enzymes
  - Pharma proteins and peptides
- 'My' Expertise:
  - Irritation
  - Carcinogensis
  - Sensitization
- Specific issues:
  - Potency is defined by ''number of patients'' not ''concentration''
  - Hazard and risk assessment ''relative'' to historical data

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Outline of the presentation

- Ensuring safety of technical enzymes without using animals
  - Historical data, data waiving and read-across
  - Solid basis for developing ITS for relative hazard identification
- Future hazard identification and risk management of industrial enzymes:
  - Computational approaches
  - *In vitro* characterization
    - Implementation of the Sens-it-iv toolbox
  - Integration of data
- Publicity:
  - Congress on In Vitro Sensitization

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

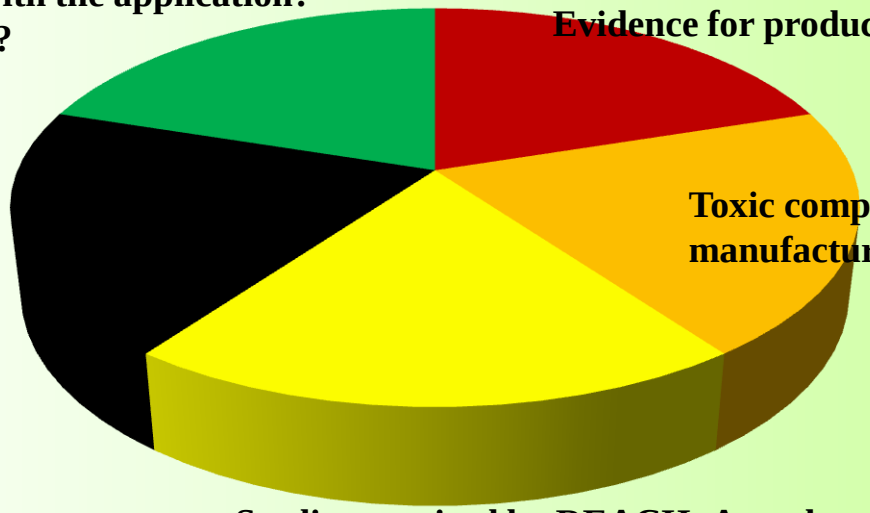
A proposed strategy for ensuring safety of detergent enzymes without using animals in the context of REACH

SIXTH FRAMEWORK PROGRAMME

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Risk assessment of industrial enzymes in the past

- Any specific risk associated with the application?
- Measures to reduce exposure?



SIXTH FRAMEWORK PROGRAMME

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Historical data, data waiving and read-across in the REACH context

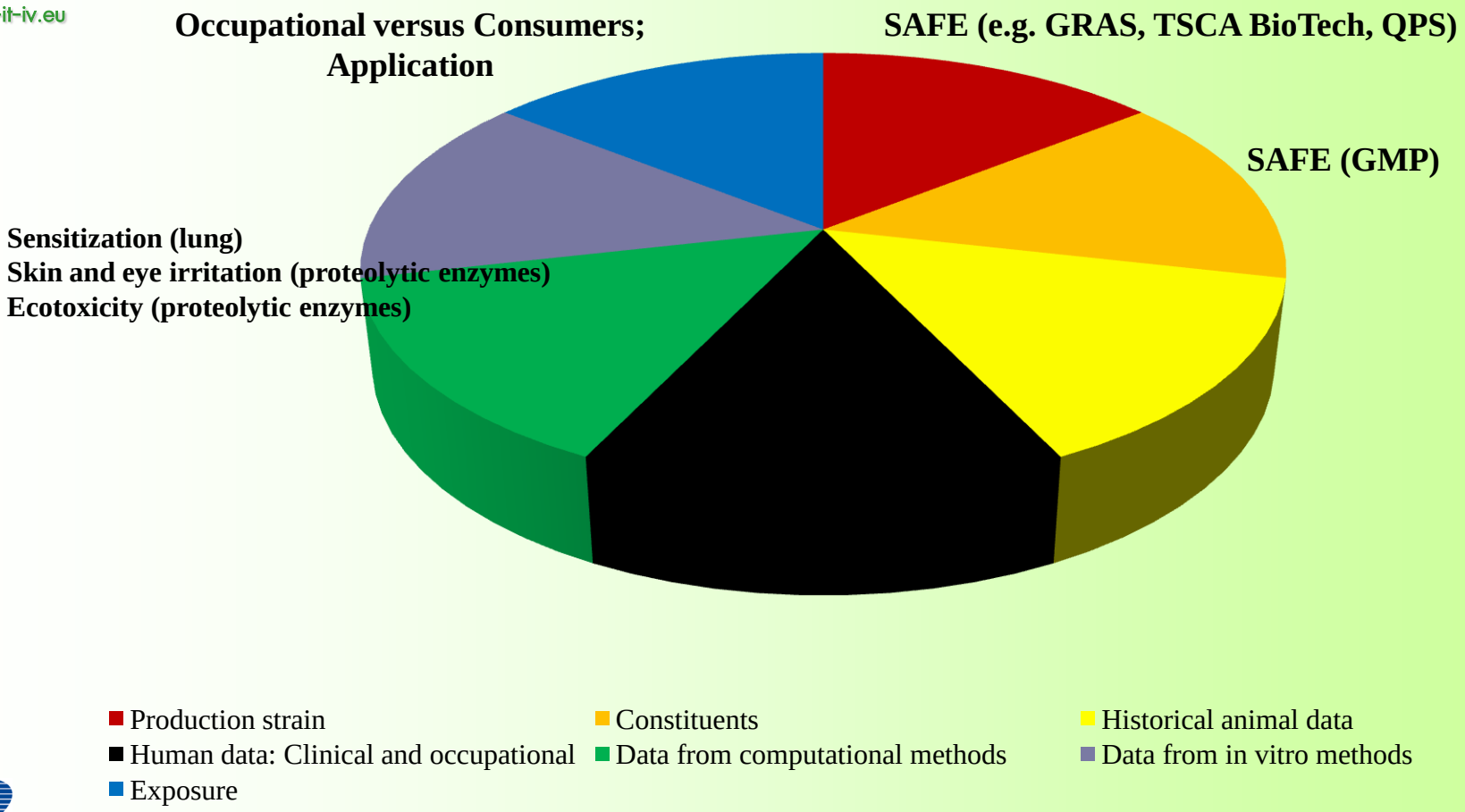
- 40 of experience and substantial documentation on
  - The safety of production strains
  - Good standard manufacturing processes
  - Characterization of the enzyme test materials
  - High quality studies for al relevant (REACH) endpoints
    - *In vivo* as well as *in vitro* studie
  - Measurements for eliminating exposure (granulates)
- Solid basis for applying read-across and data waiving without compromising safety (applied for REACH registration)
- Solid basis for development of ITS for relative hazard identification (product development and safety)

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Future strategy for ensuring safety of industrial enzymes without using animals

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Future hazard identification strategy



1. Computational approaches:

From the protein point of view …

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

The computational approach for assessing protein characteristics

- Size (1-40 kDa) and structure (?)
- Physico-chemical characteristics
  - Hydrophobicity/-philicity; sheets; loops; turns; flexibility; accessibility; ...
- Specific sequences that are recognized by T and B lymphocytes (epitopes)
  - Linear (T, B) or conformational (B)
  - Databases and prediction tools available
- Impact of the protein on the viability/functionality of mammalian (human) cells and cellular processes (under construction)
  - Enzyme activity; substrate binding (incomplete); receptor-ligand like interactions

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Comparison of the predictivity of various prediction models (B cell epitopes)

Protein structure	Prediction model		PPV (%)
Primary and secondary	Single scale	hydrophilicity	48-61
		polarity	54-58
		accessibility	50-58
		flexibility	50-71
		turns	34-72
	Combination of scales		55-65
	Machine learning tools	ABCpred, Bepi-pred	60-68
		SPA	<35-65
Primary and 3D-structure		Structural similarities	Considered too low
3D-structure	Combination of scales (see above)	CEP, DiscoTope, BEpro	66-71
	Using amino acid seqences and motifs	Pepitope	41-54
		Pep-3D-Search	70
		EMT	79-100

Roggen (2008) In *Immunogenicity of Biopharmaceuticals (van de Weert M and Møller EH)* pp 75-95, AAPS Press
Roggen (2008) Drug Discovery Today: Technologies 5, 49-55

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

2. Cellular processes:

Implementation of the selected tests from the Sens-it-iv toolbox

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Overall objective of the *Sens-it-iv* project

- Development of tools that are ready for prevalidation.
  - Physiologically relevant
    - Cell culture conditions supporting relevant cell phenotype, cell-cell interactions and cell-compound interactions
  - Mechanism of action
    - -omics
  - Addressing specific questions
    - Solid knowledge about *in vivo* processes required
  - SOP(s)
    - Including detailed performance criteria
- Tools that can be incorporated in ITS

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

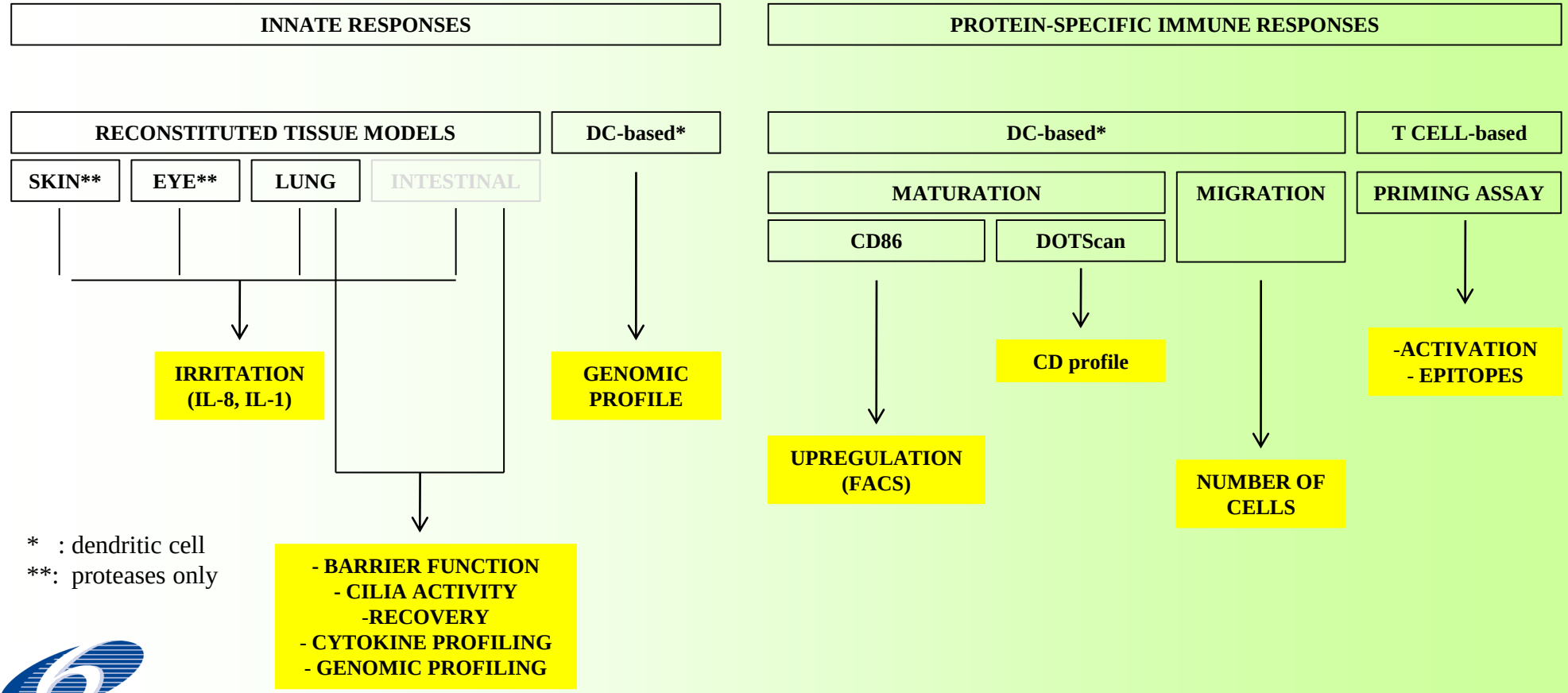
''The Sens-it-iv Toolbox''

	Protein binding
Keratinocytes	**NCTC2544 test**
	Human reconstituted skin
Lung EC	**Precision cut lung slices**
	Human reconstituted alveolar epithelium *
	Human reconstituted bronchial epithelium *
	Specific sensitizer genomic profil *
DC	**Genomic Allergen Rapid Detection (GARD) test ***
	Maturation #1 (CD86, CD54, IL-8, …) *
	Maturation #2 (DotSCan) *
	Migration *
T-cells	**Primary T-cell stimulation ***
Bioactivation	**Neutrophil - THP-1 metabolization tests**
	Proteomics marker profile (combined list)

SIXTH FRAMEWORK PROGRAMME

Measuring the impact of a protein on cells and cellular processes *in vitro*

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

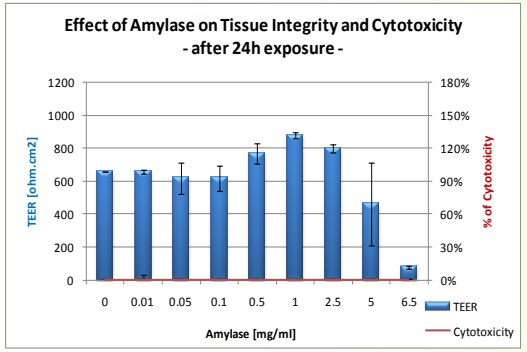


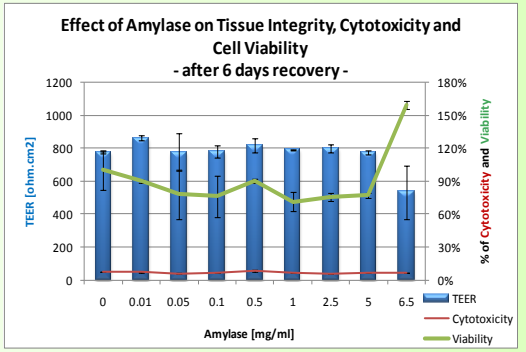
* : dendritic cell
**: proteases only

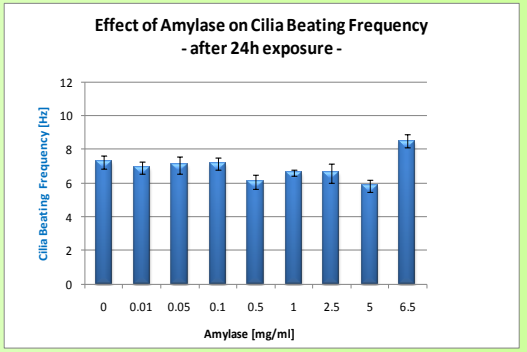
SIXTH FRAMEWORK PROGRAMME

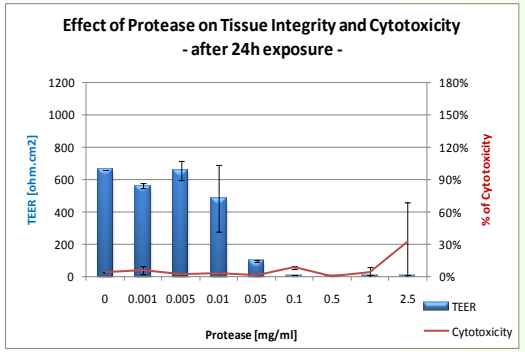
LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

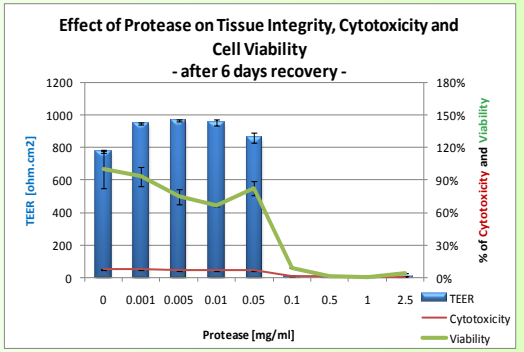
Reconstitiuted human bronchial tissue: Barrier function, cilia beating and recovery

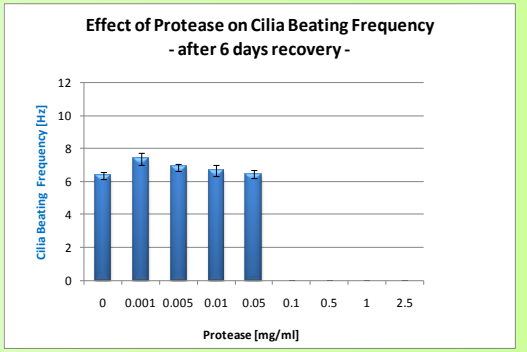










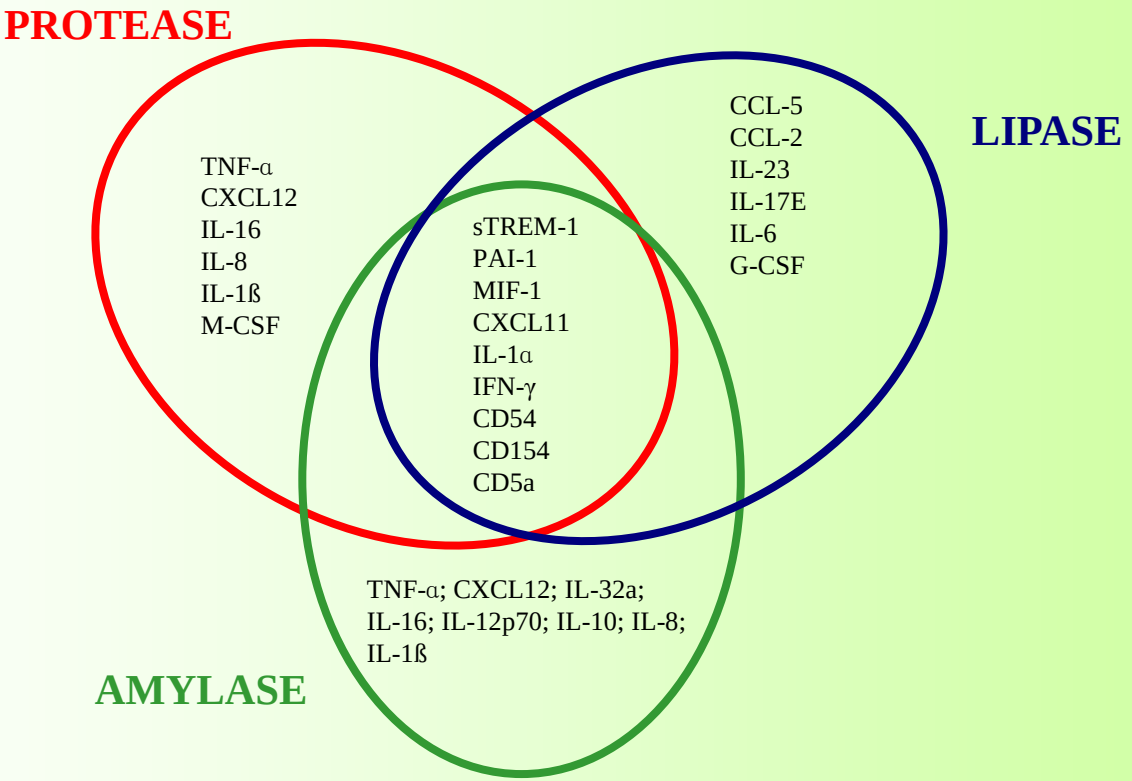


Collaboration with Epithelix

SIXTH FRAMEWORK PROGRAMME

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Reconstitiuted human alveolar tissue: Cytokine profiling

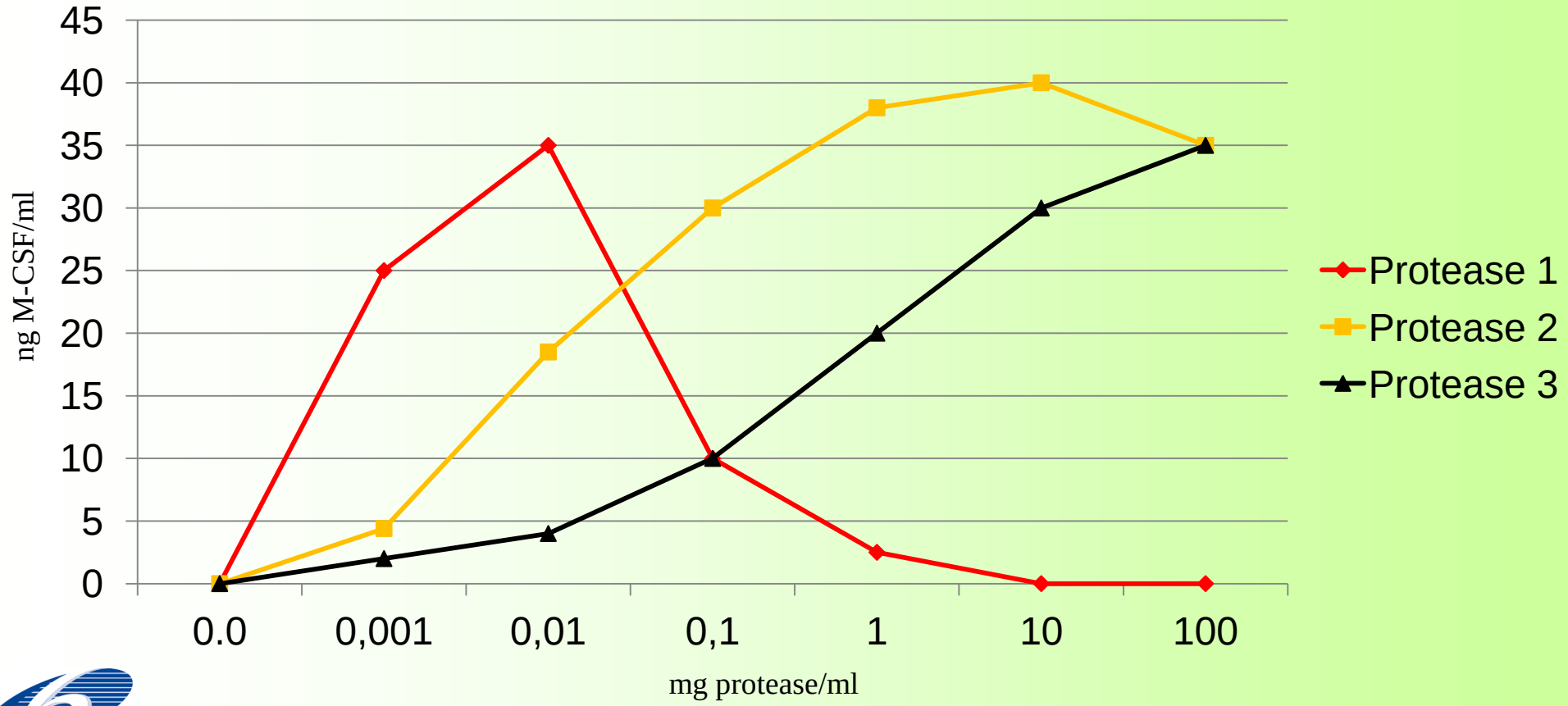


Collaboration with Mainz University

SIXTH FRAMEWORK PROGRAMME

Ranking of enzymes according to potential sensitizing potency: an example

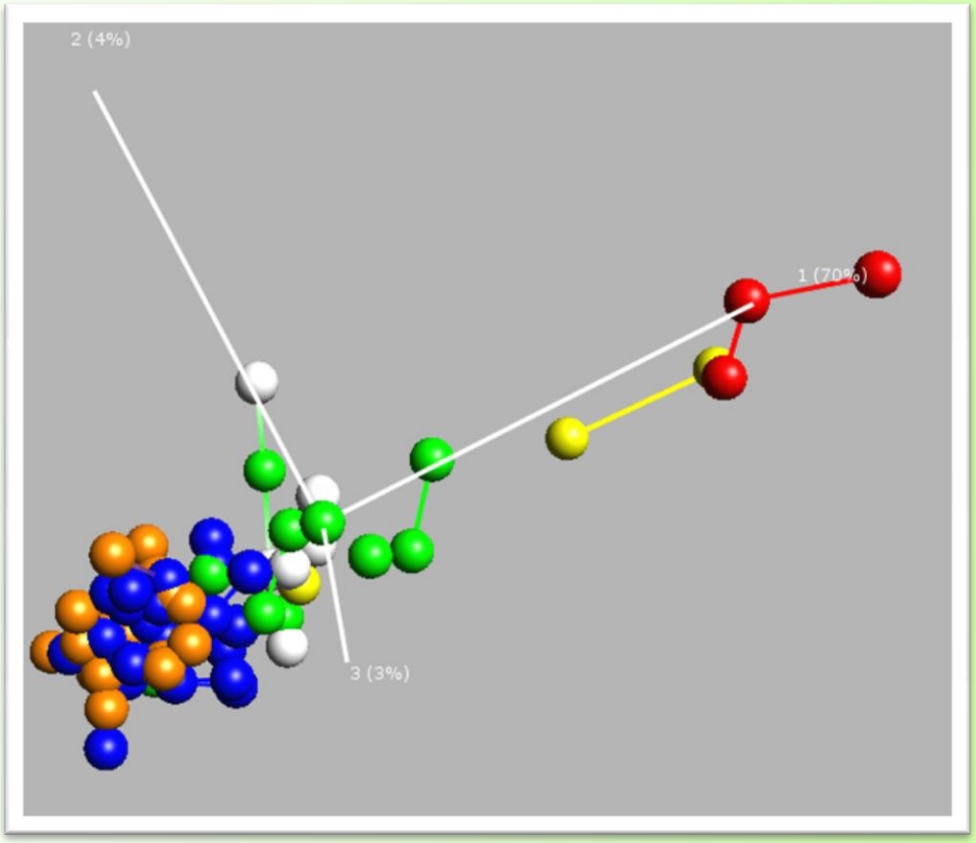
LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu



SIXTH FRAMEWORK PROGRAMME

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Genomic Analysis of MUTZ-3 Cells after exposure



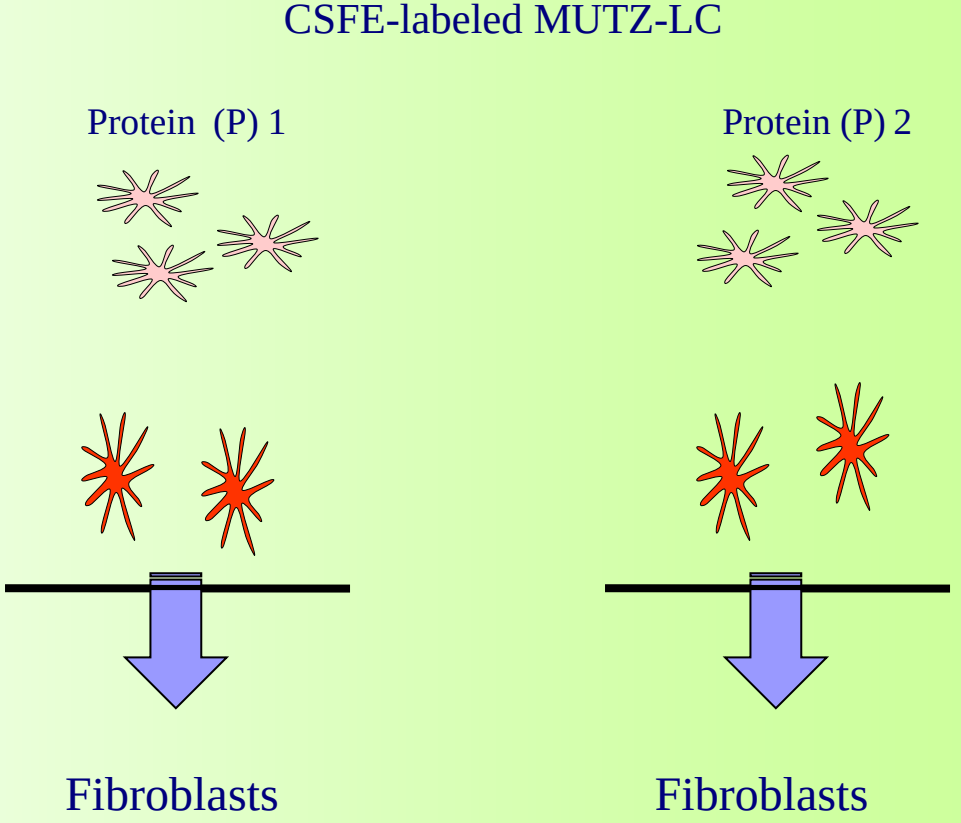
C. Borrebaeck et al. Lund University (S)

SIXTH FRAMEWORK PROGRAMME

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Transwell DC migration assay: the principle

The read-out of the assay is the number of cells migrating towards the lower compartment.



LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Integration of the data is ongoing

Enzyme activity	Variant / class	In vivo data		Computational		Innate immune responses					Adaptive immunc responses		
		Animal	Human	B cell	T cell	Barrier function (mg/ml)	Cilia beating (mg/ml)	Recovery	Cytokine profile (mg/ml)	Genomic profile	DC maturation	DC migration	T cell priming
Protease	1	**Animals** Guinea pig, Rat, Mouse Serological data Immunochemcial characterization **Humans** Clinical studies, Occupational data Serological data		Epitope lists available Overlaps and differences identified		0.01	0.01	no	0.001	Analysis in progress	Analysis in progress		Epitopes identified
	2					0.1	0.1	no	0.1				
	3					10	10	yes	10				
Amylase	A			Epitope lists available Overlaps and differences identifed		10	10	yes					
	B					No effect	No effect	--					
Others (Lipase)				Epitope lists available		No effect	No effect	--					

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Integration of the data is ongoing

Enzyme activity	Variant / class	In vivo data		Computational		Innate immune responses					Adaptive immunc responses		
		Animal	Human	B cell	T cell	Barrier function (mg/ml)	Cilia beating (mg/ml)	Recovery	Cytokine [illegible] (mg/ml)	Genomic profile	DC maturation	DC migration	T cell priming
Protease	1	**Animals** Guinea pig, Rat, Mouse Serological data		Epitope lists available		0.01	[illegible]	[illegible]	0.001	Analysis in progress	Analysis in progress		Epitopes identified
	2			Overlaps [illegible] differe[illegible] identifi[illegible]		[illegible]	0.1	no	0.1				
	3					10	10	yes	10				
Amylase	A	Immunochemcial characterization		Epitope lists available		10	[illegible]	yes	[illegible]				
	B	**Humans**		Overlaps and differences identifed		No effect	No effect	[illegible]	[illegible]				
Others (Lipase)		Clinical studies, Occupational data Serological data		Epitope lists available		No effect	No effect	--					

Seems to be confirmed by animal studies

Also observed in animals and humans

SIXTH FRAMEWORK PROGRAMME

LSHB-CT-2005-018681
2006-2010
www.sens-it-iv.eu

Summary

- Set-up still 'unstable'
  - Sufficient/adequate testing?
  - Avoid 'overkill' for the sake of time and money
  - Integration of historical data, computational data and in vitro data to be optimised
  - The weight of each input still to be established
- Ambition to be able to perform 'relative' hazard identification and risk assessment
- Learnings from industrial enzymes should help to establish testing strategies for BioPharmaceuticals