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Generation of a humanized Fas Ligand mouse and its application in pemphigus: a new platform for the study of PC111 antibody

Roberta Lotti[1,2], Alessandra Marconi[1,2], Elisabetta Palazzo[1], Marika Quadri[1], Emmanuel Sotirakis[3], Vincent Dubus[3], Antonino Amato[2], Brydon Bennett[2], Carlo Pincelli[1,2]

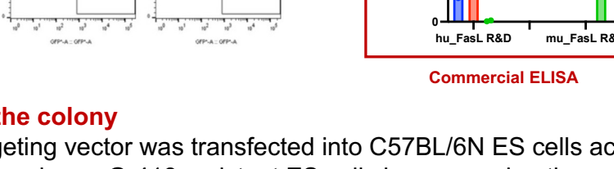
1. DermoLab, UNIMORE, Modena, Italy. 2. PinCell s.r.l, Milano, Italy. 3. genOway SA, Lyon, France

INTRODUCTION

	PEMPHIGUS
Characteristics	Autoimmune disease, with blisters and erosions of skin/mucosae; diagnosed in middle age
Course of disease	Chronic, debilitating and life-threatening with severe impact on QoL Overall mortality 5-15% due to side effects (3x controls)
Epidemiology	Prevalence 1,92/10,000 worldwide Target population ~300,000 patients worldwide
Approved Treatment	Rituximab plus steroids (2020)
Unmet Medical Needs	Relapsing (up to 60%) and refractory patients Severe side effects due to extended immune-suppression

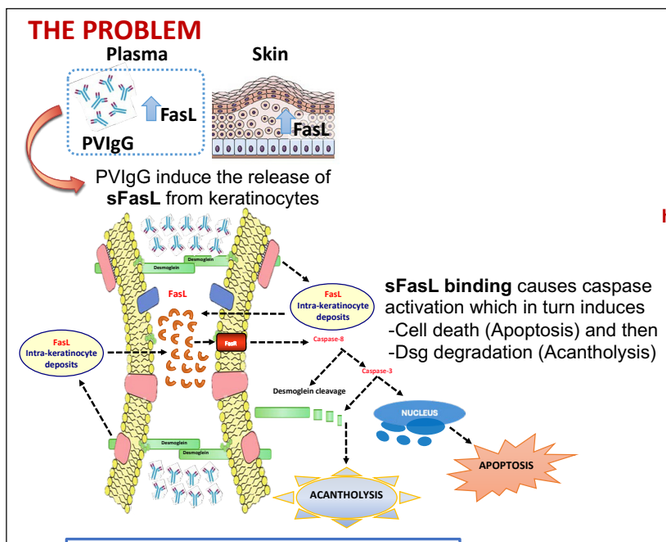
ROLE OF FASL IN PEMPHIGUS

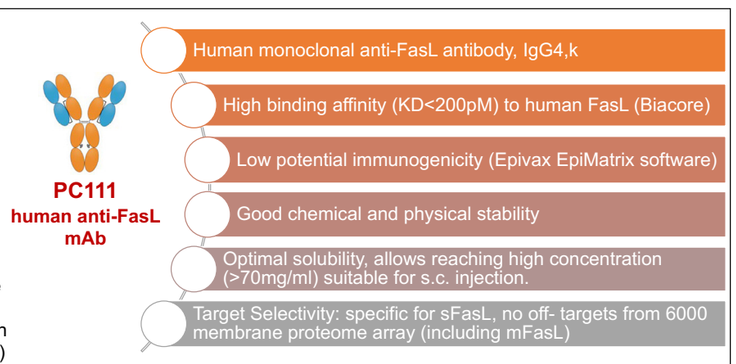
- Pemphigus autoantibodies (PVIgG) trigger the release of FasL from keratinocytes (*Wang et al, 2004*) and high levels of FasL measured in patients' sera (*Puviani et al, 2003)*
- FasL-silenced keratinocytes are protected from PVIgG-induced desmoglein-3 (Dsg3) cleavage and activation of caspase-8 induced in vitro. Moreover caspase-3 is not activated in siFasL keratinocytes upon PVIgG treatment (*Lotti et al, 2018*).
- Soluble FasL is essential for the process of blister formation in pemphigus (*Lotti et al, 2018*).



- Anti-murine FasL antibody prevents blister formation and apoptosis activation in the passive transfer pemphigus mouse model (*Lotti et al, 2018*).

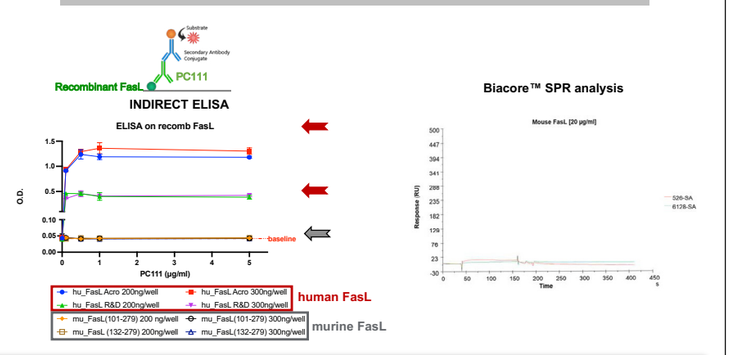
RATIONALE and AIM





BUT

No Cross-reactivity binding to mouse FasL



AIM: to develope a humanized FasL (hu_FasL) mouse, in order to test the efficacy of PC111 in a human in vivo setting

RESULTS

1. Generation of the new hu_FasL mouse

The FasL humanized model was generated by **gene swapping**: the human FASL genomic region (in pink) spanning the whole encoding-extracellular region was inserted in frame with mouse exon 1 downstream of mouse residues (YQLF) encoding for the C-terminal transmembrane domain, consequently, the model expresses a **chimeric FASL protein (mIC-mTM-hEC, red box)** while the insertion resulted in deletion of the mouse counterpart genomic region. A loxP-flanked Neomycin resistance cassette, was inserted downstream of the human FASL exon 1 and removed on the final model leaving a single loxP site.

2. Validation of expressing cells

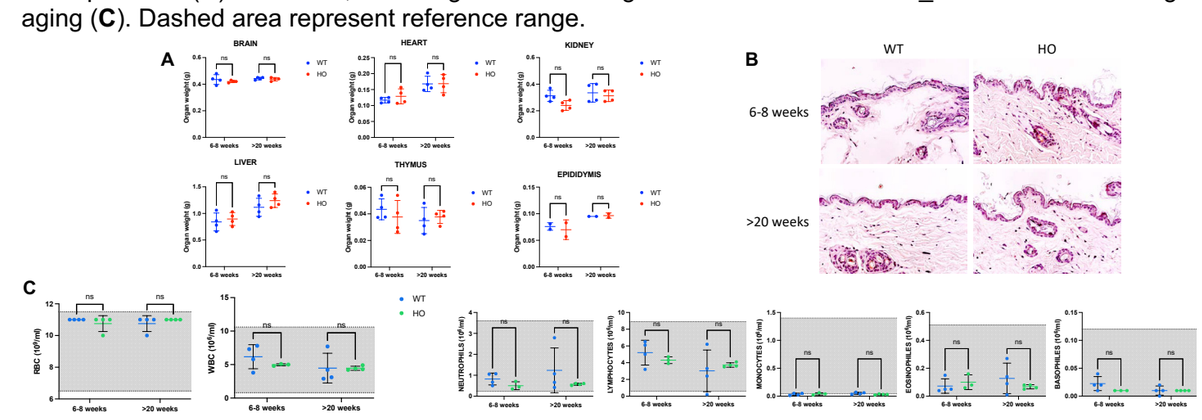
Quantification of specific sFasL in the supernatant of GFP-expressing stable RAW264.7 cells (**A**), either by a commercial ELISA (R&D Systems) or by PC111 indirect ELISA (**B**).

3. Generation of the colony

The linearized targeting vector was transfected into C57BL/6N ES cells according to genOway's electroporation procedures. G-418 resistant ES cell clones carrying the recombined locus were identified by PCR while the integrity of the humanized cassette was confirmed by sequencing analysis (data not shown). Recombined ES cell clones were microinjected into C57BL/6N blastocysts, and gave rise to male chimeras with a significant ES cell contribution. Breeding was established with C57BL/6N mice expressing Cre recombinase mice, to produce the FASL humanized mice devoid of Neomycin cassette (**C57BL/6NCrl-FasLtm1.1(FASL)Geno**).

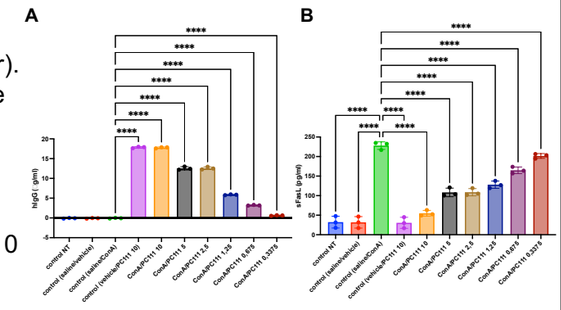
4. Characterization of the homozygous progeny during aging

We analysed the homozygous progeny (HO) and compared it to wild type (WT) counterpart, during aging. We harvested some organs and compared their weight in the the two populations (**A**), with not significant differences (ns). No histological changes of any tissues in hu_FasL mice. Representative pictures of skin are reported in (**B**). Moreover, no changes of circulating cells were detected in hu_FasL mice also during aging (**C**). Dashed area represent reference range.



5. PK/PD studies in hu_FasL mice

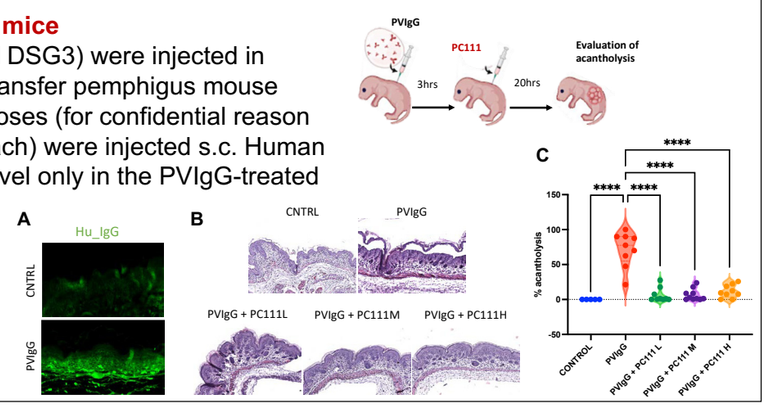
hu_FasL HO mice were injected with 12 mg/kg dose of Con A, 2 hrs after injection sFasL is elevated (**B**, green bar). Levels of PC111 in the blood at 2 hours from different dose groups as measured by a human IgG specific ELISA. A near linear increase in human IgG from 0.33 mg/kg to 10 mg/kg of PC111(**A**). The same plasma samples were analyzed for sFasL. Administration of PC111 showed a near linear decrease in human sFasL from 0.33 mg/kg to 10 mg/kg of PC111 (**B**). (****p<0.0001, Dunnett adj, n=3)



6. PC111 PoC in pemphigus Hu_FasL mice

PVIgG from patients (against DSG1 and DSG3) were injected in hu_FasL neonatal mice in the passive transfer pemphigus mouse model. 3hrs after PVIgG, three PC111 doses (for confidential reason indicated as Low, Medium, High, 10X each) were injected s.c. Human IgG were detected at the desmosome level only in the PVIgG-treated group (**A**).

We analysed the acantholytic effect at the histological level (**B**). PC111 blocks blister formation (with approximately 90% inhibition), also at the lowest dose (**C**). (****p<0.0001, Dunnett adj, n=9)



IN SUMMARY

- PC111 shows **no binding to murine FasL**
- **Hu_FasL mouse platform** was generated
- **No histological or hematological changes** during aging of hu_FasL mice
- **PK/PD study** was completed on the hu_FasL mice
- PC111 **efficacy was confirmed** in neonatal hu_FasL mice using the PVIgG passive transfer model

KEY REFERENCES

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