# DRUG T CELL REACTIVITY IN DELAYED TYPE HYPERSENSITIVITY – EVALUATION WITH CYTO-LTT (5 CYTOKINES PANEL ASSAY)

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# AGENDA

- **Drug hypersensitivity:**
  **Immunogenicity <u>beyond biologicals</u>**
  - Drug *hypersensitivity* is clinically heterogenous
  - Drug *immunogenicity* is not limited to biologicals
  - Clinical *severity* correlates to how T cells are activated
- **T cell immunogenicity assay (Cyto-LTT)**
  **Identification of culprit drug and cross-reactivity**
  - <u>Case 1: Small molecule drug</u>
    e.g. β-lactams amoxicillin & cefuroxime
  - <u>Case 2: Biological drug (human antibody)</u>
    e.g. dupilumab
- **Conclusions**
  - Multi-cytokine assay (Cyto-LTT) *improves test sensitivity*
  - Utility in clinical *diagnostics* & *pre-clinical assessments*

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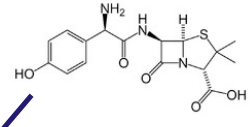
# One substance → various pathologies

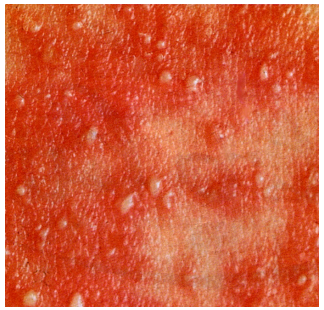
**Maculopapular Exanthema**

CD4+ > CD8+
IL5, Cytotoxicity, IFNγ

*Yawalkar et al, JACI 2000*
*Hennino et al, JACI 2011*

**Amoxicillin**





**AGEP**

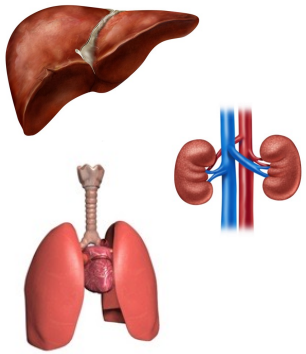
Neutrophils >>>CD4+
IL8, IL36γ

*Meier-Schiesser B. J. Invest. Dermatol 2018*

**DRESS**

CD4+ >> CD8+
IL5, Cytotoxicity

*Miyagawa et al,*
*Int J Mol Sci 2021*



**SJS TEN**

CD8+, NK+ >>CD4
GzB, GL
Terminally differentiated CTL

*Villani & Rozieres et al. Sc. Advances 2021*



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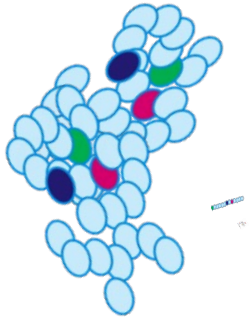
# Immune reaction to Xenobiotics

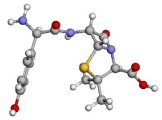
## How can small molecules be recognised by immune receptors?

Biological
17,000 Da



Peptide 13 aa
1,000 Da

Small molecule
400 Da



**Drug size**

Too small to be considered immunogenic by the immune system?

How can reactivity be explained?

i. Hapten concept
ii. p-i concept

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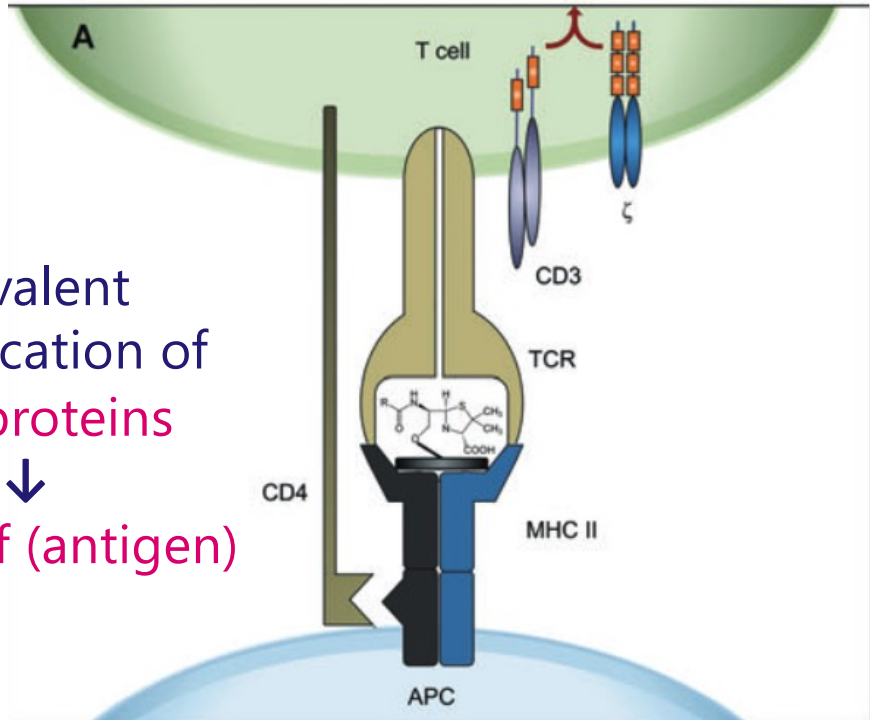
# How does the immune system “see” small molecule drugs

**Hapten concept**

Covalent binding




Covalent modification of self-proteins

↓

non-self (antigen)

Pre-clinical assessment:

Chemistry of drug candidate

Identification of possible off target sites (HLA/TCR)

Adam *et al* (2011). *Br J Clin Pharmacol* ***71(5)***



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# Severe symptoms relate to p-i mechanism

β-lactams [1]
MPE patients TCC → Hapten
DRESS patients TCC → p-i mechanism

Carbamazepine [2,3]
SJS/TEN → p-i mechanism

Sulfonamides [4]
MPE, DRESS, SJS → p-i mechanism

Allopurinol [5,6]
Metabolite (Oxypurinol) responsible for reactivity
HLA risk allele (HLA-B*58:01)


(1) Wuillemin *et al* (2022) *Frontiers in Allergy* **3**
(2) Wei *et al* (2012) *JACI* **129(6)**
(3) Jaruthamsophon *et al* (2023) *Chem Res Toxicol* **36(5)**
(4) Pichler & Brüggen (2022) *Allergy* **78(1)**
(5) Yun *et al* (2013) *Clin Exp Allergy* **43(11)**
(6) Yun *et al* (2014) *J Immunol* **192(7)**




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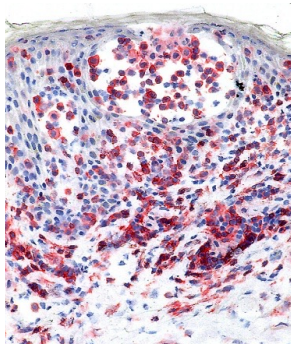
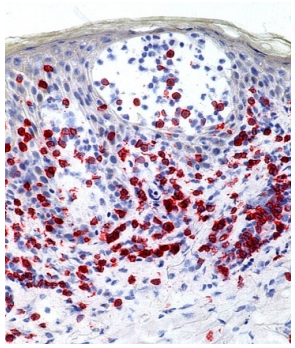
# Direct mediation of symptoms by T cells infiltrating into organ lesions

**Cutaneous lesions**

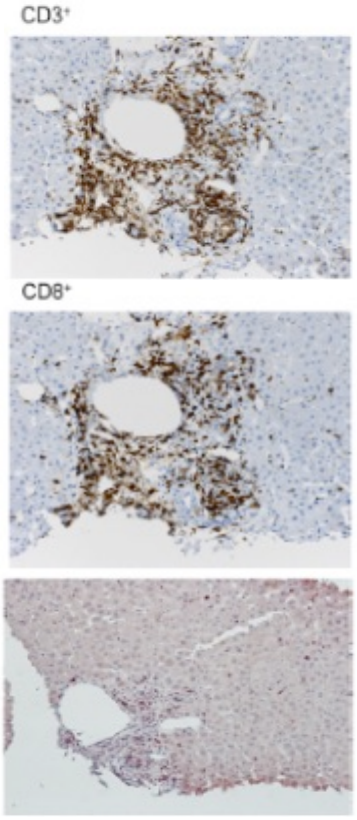
**AGEP**
Acute Generalised Exanthematous Pustulosis



CD4 staining



CD8 staining

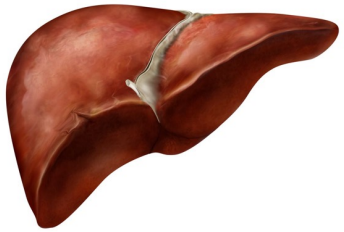



CD3+

CD8+

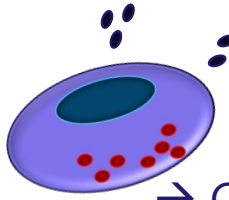
TIA-1+

**Internal lesions**



e.g. liver infiltration

Cytotoxic properties



T cells:
→ Cytokines
→ Cytotoxicity

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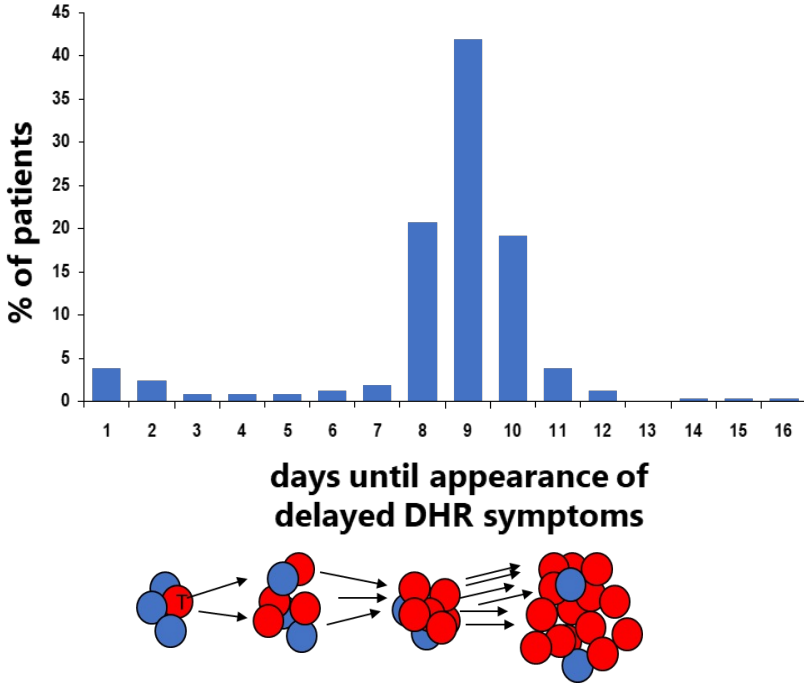
# Drug-specific T cell activation

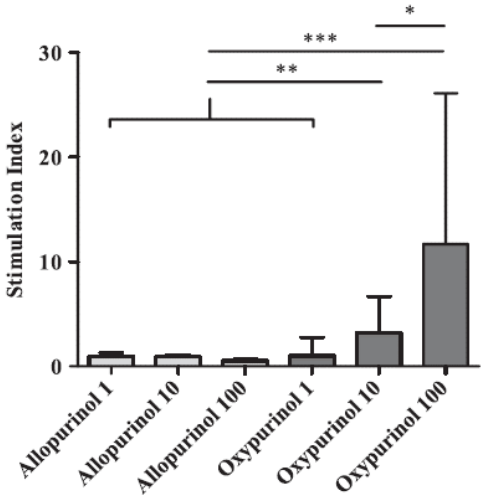
## *In vivo*

N=270 delayed DHR patients to gemifloxacin




Schmid *et al.* (2006). *Curr Pharm Des* **12(26)**

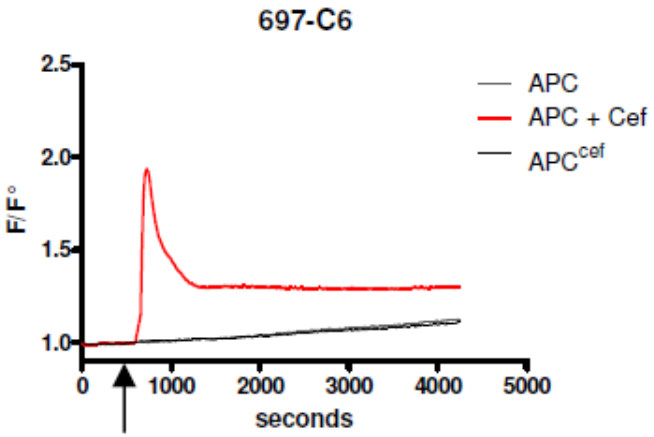
## *In vitro* proliferation




Specificity;
Drug metabolite

Yun *et al.* (2013). *Clin Exp Allergy* **43(11)**

## Calcium influx




Rapid direct activation
(p-i mechanism)

Wuillemin *et al.* (2022). *Frontiers in Allergy* **3**



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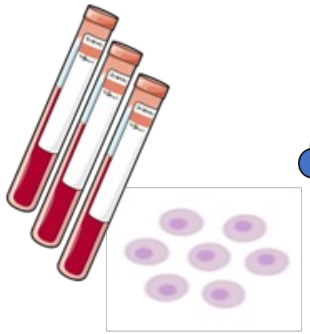
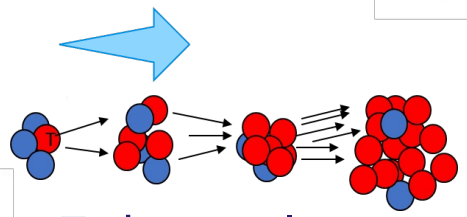
# Cytokine release T cell assay (Cyto-LTT)

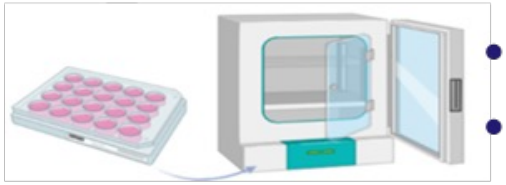
**Isolate PBMCs from whole blood**





7 days culture

**Immunophenotyping Assays**



- Cell culture (**Cyto-LTT**)
- **Cytokine** profiling

***Type I***
IFNγ

***Type II***
IL-5 & IL-13

***Cytotoxic***
Granzyme B (GzB)
Granulysin (GL)

| Stimuli | |
|---|---|
| Neg. | Cell culture medium |
| Pos. | Pokeweed mitogen |
| Pos. | Tetanus toxoid |
| Drugs | tested in 3 – 5 concentrations |

Thoo *et al.* (2024). *Allergy* - submitted
Lochmatter *et al.* (2009). *Allergy* **64(9)**

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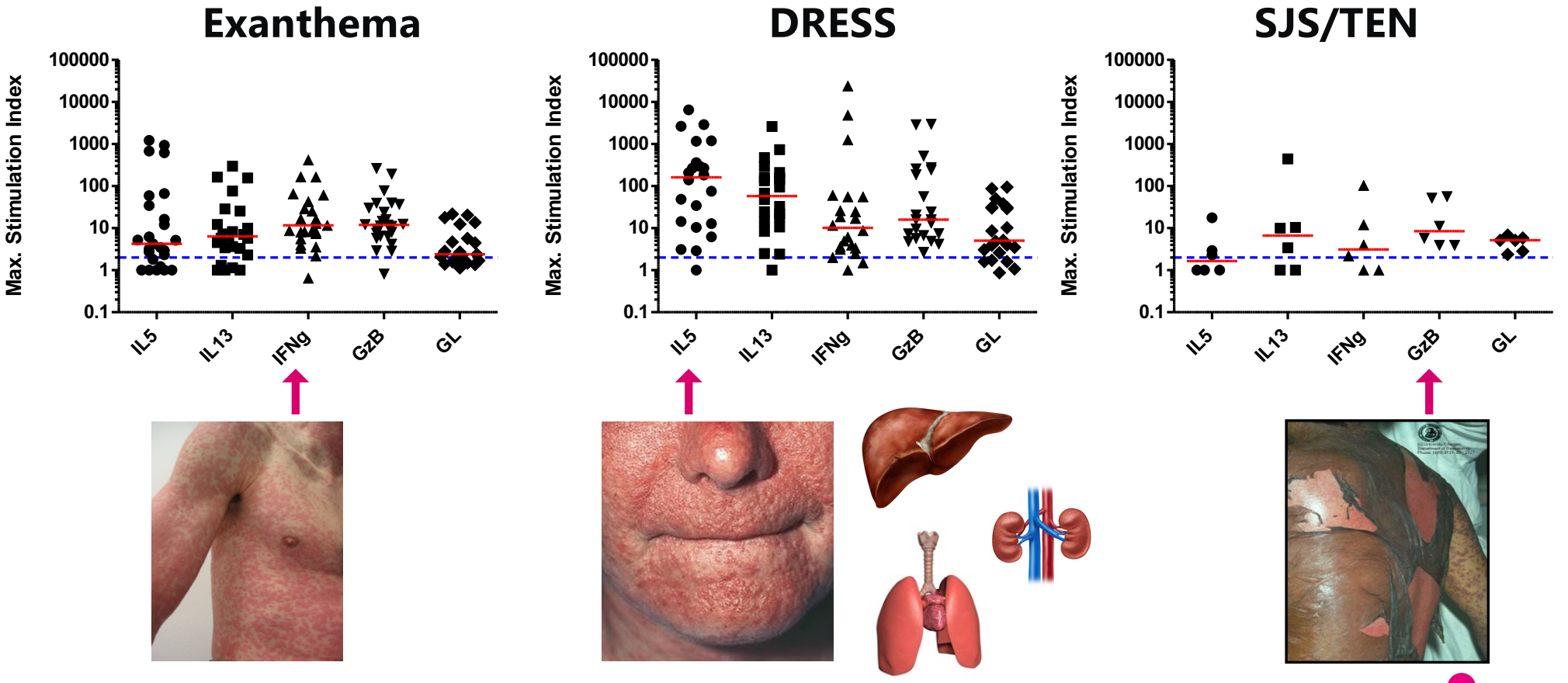
# Cytokine pattern and clinical symptoms



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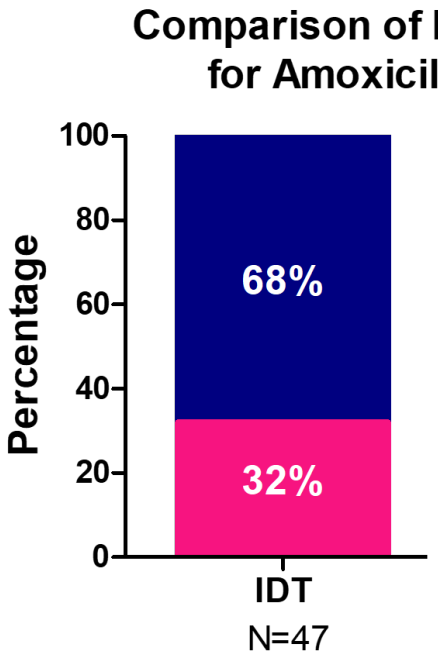
# Case 1: Small molecule drug
## Cross-reactivity: Amoxicillin & Cefuroxime

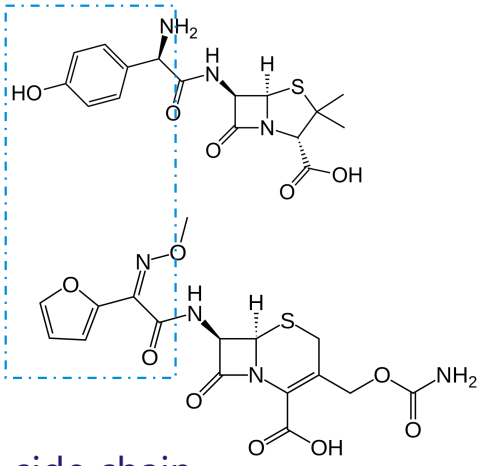



**IDT = Intradermal skin test**

Cyto-LTT > IDT
higher sensitivity to detect drug sensitisation

**8 / 43** of the Amoxicillin CytoLTT + were **also Cefuroxime CytoLTT +**

**Test result**
Positive
Negative



← Amoxicillin

← Cefuroxime

R1 side chain
Different; low cross-reactivity but *possible*

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# Case 2: Biologic drug
## e.g. Dupilumab

DHR Symptoms; <u>not infusion reactions</u>
e.g. Rash, erythema nodosum

| Reinsubstanzen | IL-5 | IL-13 | IFNg | GzB | GL |
|---|---|---|---|---|---|
| Kontrolle | Positiv (2) | Positiv (2) | Positiv (2) | Positiv (2) | Positiv (1) |
| Pokeweed Mitogen | 59,45 | 381,39 | 3600,00 | 621,31 | 8,67 |
| Tetanus Toxoid | 6,34 | 10,42 | 178,39 | 38,07 | 1,80 |

- Sensitisation only to dupilumab
- **<u>not</u>** to excipient PS80 (documented allergen in some individuals)
- No sensitisation to different asthma drug (omalizumab)

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# Take home messages

1. **Off target drug binding to immune receptors** can "fool" the immune system into activation **(p-i concept)**.

2. Cyto-LTT (cytokines) allows identification of culprit drugs with association to clinical diagnosis (e.g. MPE vs DRESS)

- ✓ Learn from patients (diagnostics)
- ✓ Improve pre-clinical assessments

3. Long *in vitro* culture of drug in naïve individuals for risk assessment.

4. Utility during risk assessment:
   - Stimulation mechanism – p-i mechanism *vs* haptenization.
   - HLA/TCR restriction: risk can be diminished.
   - Cross-reactivity (new drug candidate *vs* existing drug).
   - Confirmation of *in silico* docking data i.e. drug binding to which molecules / cells.
   - Clinical surveillance (Rare risk factors – Pharmacovigilance evaluations).

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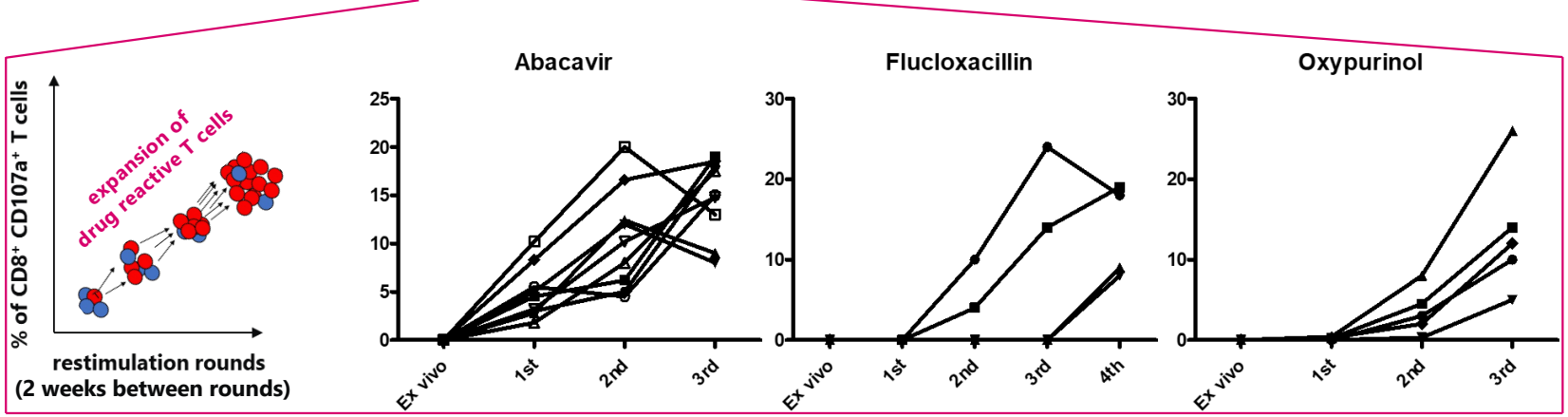
Example risk assessment process

# *How to study drug T cell reactivity?*
# *In vitro* priming of drug reactive T cells

**Identify reactive drug & risk factors (patients)** → **Expand T cells *in vitro* from naïve blood donors (with risk factor)** → **Assessment of T cell clones** → **Compare new drug / derivative with previously reactive drug** → **New candidate: Immunogenicity reduced?**




e.g. assessment of IL-5 release by T cells


- Naïve blood donors
- Presence of risk factors (HLA allele, TCR repertoire, e.g. abacavir, oxypurinol)
- Several restimulation rounds with IL-2 (every 2 weeks ⟶ months)

- **Utility**:
  - Evaluate new drug candidate *vs* existing drug (cross-reactivity risk)
  - Mechanistic evaluations: p-i / hapten → correlation to clinical severity risk


Wuillemin *et al* (2013) *J Immunol* **190(10)**
Adam *et al* (2014) *PloS One* **9(4)**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*Thank you!*

Delighted to discuss further:

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